Supplemental Material

For

A woolly mammoth (*Mammuthus primigenius*) carcass from Maly Lyakhovsky Island (New Siberian Islands, Russian Federation)

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S1. Methods

Analytical methods utilized during this study are as diverse as the subjects under investigation. The earliest stages of excavation and exposure were done without elaborate precautions to avoid contamination because the degraded parts of the specimen had clearly already experienced extensive environmental exposure. Nonetheless, all sampling for hematological, histological, microbiological, and other compositional analyses was conducted using latex gloves, sterile tools, and sterile sample containers. Other methodological details are summarized below.

S1.1. Radiocarbon dating

Two samples of the Malolyakhovsky mammoth, one of bone and one of hair, were assayed to determine age by accelerator mass spectrometry (AMS). For bone (rib), collagen was extracted following an improved version of the procedure originally developed by Longin (1971). The hair sample underwent standard AAA (Acid-Alkali-Acid) treatment (Mook and Streurman, 1983).

The purified datable fraction of each sample was combusted using an Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer (IsoCube/IsoPrime). This IRMS provided the stable isotope ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$ as well as the C and N yields.

For ^{14}C analysis, part of the CO_2 obtained was routed to a cryogenic trap to collect samples for further processing. The carbon from the CO_2 was converted to graphite powder (Aerts et al., 2001), and the graphite was pressed into target holders for the ion source of the AMS. The AMS measured the $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratios of the graphite (van der Plicht et al., 2000). From these values, the conventional ^{14}C age was calculated. ^{14}C ages are reported in BP by convention. This convention is based on the Libby half-life value, oxalic acid as a reference, and correction for isotopic fractionation using $^{13}C/^{12}C$ (Mook and van der Plicht, 1999).

For absolute dates, conventional ^{14}C ages were calibrated to calendar ages. This was done using the calibration curve IntCal13 (Reimer et al., 2013). Calibrated ages are reported in cal BP (calendar age relative to 1950 AD).

S1.2. Surface-scanning

To capture data on external surface configuration, we made selective use of a Creaform VIUscan (ZScan 700 CX) laser scanning digitizer to generate digital 3D models with a resolution of about 0.1 mm. This scanner also captures image data with which it generates a texture map that can be added to the surface model to yield an approximately photorealistic model.

The modeling process requires self-adhesive reflective markers to be attached to the surface in an irregular pattern; the distances between four or more of these markers are used as a unique location signature to allow the digitizer to add new scan data to an existing model. The 3D scanner measures a large number of points on an object's surface and outputs a point cloud. The point cloud represents the set of data points that the device has measured. In a three-dimensional coordinate system, these points are defined by *x*-, *y*-, and *z*-coordinates and collectively represent the external surface of an object.

To convert point clouds into three-dimensional surfaces we used the software ZScan. The program Rapidform was used to connect separate scans, fill cavities and remove noise. This program also allowed us to measure some parameters of the scanned objects. The program 3DsMax was used to produce the photorealistic texture-mapping of the tusk models.

S1.3. Computed tomography

For non-destructive analysis of the 3D structure of body parts, we used computed tomography facilities at the Yakutsk Hospital. These included a Toshiba Aquilion, used for soft and hard tissues, or body parts that included both, and a Siemens Somatom Definition AS scanner, used only for the tusks. Each of these scanners was operated at 120kV, but the Aquilion typically ran at 500ms integration time, while the Somatom Definition ran at 1000ms integration time. Beam current for the Aquilion ranged from 80mA, using a protocol for soft tissue or a mix of soft and hard tissue, to 200mA, using a protocol for "extremities" which we selected for imaging density variation within the tusks. Beam current for the Somatom Definition was 75mA. Voxel resolution was in some cases as good as 0.488mm x 0.488mm in x-y and 0.3mm in z, but was more often 0.933mm x 0.933mm in x-y and 0.6mm in z.

Each tusk of the Malolyakhovskiy mammoth is over two meters long and strongly curved. Their size and curvature prevents them from fitting through a standard medical CT scanner in a single scan. We therefore scanned each tusk in three parts. To assist in splicing the resulting data together into a single model, we glued wooden blocks onto the side of each tusk in multiple places, so that several such markers were included in each scan.

A basic feature of the pattern of variation in X-ray attenuation of tusk dentin in specimens like this is a cyclic pattern that we recognize as having formed over the course of dentin apposition during one year, from one winter-spring boundary to the next (e.g., El Adli et al., 2016). These

annual increments in mammoths are generally thicker near the tusk axis and taper toward the dentin-cementum junction. Because of this, comparing thickness of consecutive increments requires a standard position for measurement, which we take as half-way between the axis and the cementum.

We ultimately intend to analyze the tusk CT data in full 3D. However, to expedite analysis for this study, we considered only 2D data on the longitudinal cross-sectional area of the tusk attributable to each year's apposition of dentin. This was done by constructing a curved surface following the tusk axis and imaging the pattern of variation in X-ray attenuation intersected by this surface. This pattern was saved on a flattened representation of the curved surface, and the boundaries of annual increments of dentin were traced within this plane. Areas attributed to each year were measured using NIH Image J. A second curved surface at right angles to the first (also following the tusk axis) provided a second sample of this pattern, and the areas for each year were averaged. Data were then plotted for each consecutive year in the tusk.

S1.4. Hematology

Research on hematology was done in the diagnostic laboratory of the Clinic of the Medical Institute of North-Eastern Federal University named after M.K. Ammosov (NEFU). To study the properties of fluids that we suspected might have some relation to blood, we examined swabs prepared by the classical Romanovky-Giemsa staining method for cellular elements, using an Olympus CX31 binocular microscope (at magnifications of 40X-1000X). This method allowed us to identify elements of cell nuclei by staining properties, in which acidophile elements are stained in various shades of red and basophil elements show up as purple to blue.

Hematological analysis was performed in an Adviya 2120 automated analyzer using peroxidase cytochemical reaction technology, which allowed us to detect and differentiate cellular elements in a wide range of sizes and densities (0.2 - 400 x 10^9/L). In addition, we used a two-

dimensional laser diffusion technique that allowed us to identify individual cells and determine their properties. To study hemoglobin, we used the technique of non-cyanide definition. Biochemical research on liquid samples was carried out with a Horiba ABX Pentra 400 automatic analyzer using colorimetric, photometric, turbidimetric, and potentiometric techniques to determine the concentration of individual chemicals and metabolic products to obtain information about metabolic and/or decompositional processes.

S1.5. Histology

Histomorphological and ultrastructural studies were conducted at the laboratory and clinic for Immunomorphological Research at NEFU. Tissue samples were selected from the upper, middle and lower third of the trunk of the mammoth. Histologic slides were prepared by standard techniques. Material was fixed in a 10% buffered neutral solution of formalin. Preparation of paraffin sections 3-5 microns thick was carried out on a Leica SM2010R microtome. The resulting sections for histologic survey were stained by hematoxylin and eosin, pikrofuksin on Van-Gizon, Sudan III, and the periodic acid-Schiff reaction. Microscopic examination of histologic sections was conducted on a Nikon Eclipse Ci-E compound microscope.

S1.6. Microbiology

A microbial culturomic approach, combined with metagenomic study of genetic material recovered from the carcass of the mammoth, was used to characterize the microbial communities of the mammoth's tissues and digestive tract. Samples were collected aseptically, placed into sterile containers, and stored at -20°C until analysis. For enrichments and isolations the following media were used: brain heart infusion (BHI) liquid and agar, Columbia Broth, and MacConkey agar (Oxoid Limited, UK). Single colonies representing every unique colony type were chosen from each

plate and were characterized by Gram stain, motility, and cellular morphology. Isolates were primarily identified by biochemical characteristics based on VITEK® 2 Compact (bioMérieux) panels. The taxonomic positions of selected bacteria were confirmed by 16S ribosomal DNA (rDNA) sequencing.

The 16S rRNA gene fragments were amplified and sequenced using fD1 (5'-AGAGTTTGATCCTGGCTCAG- 3') and rP2 (5'-ACGGCTACCTTGTTACGACTT- 3') primers (Weisburg et al., 1991). Sequences were aligned and percent similarities between aligned sequences were determined by using the EzTaxon (http://www.ezbiocloud.net/eztaxon) and GenBank databases (http://www.ncbi.nlm.nih.gov/nucleotide).

For 16S rRNA gene-based metagenomic analysis, total DNA from a 1-g sample of feces was extracted using the QIAamp Fast DNA stool minikit (Qiagen). PCR fragments of 16S rRNA genes were obtained using the barcoded primers with adapter sequences F515-LibL-A-MID1 5' - CCATCTCATCCCTGCGTGTCTCCGAC--TCAG—AGACGCACTC--GTGCCAGCMGCCGCGGTAA - 3' and R806-LIbL-B 5' - CCTATCCCCTGTGTGCCTTGGCAGTC--GGACTACVSGGGTATCTAAT- 3'. These were sequenced in the GS Junior Sequencing System (454 Life Sciences, Roche, Germany) according to the manufacturer's protocol. The initial taxonomic classification of these sequences was performed using the RDP classifier (http://rdp.cme.msu.edu/classifier/ classifier.jsp) (Cole et al., 2014). Cluster analysis of sequences was then performed using the program MEGAN (Huson et al., 2007).

Figure S1. Occlusal view of maxillae of Malolyakhovsky mammoth reattached to the main portion of the paired premaxillae; posterior toward top of image, anterior toward bottom, left molar on left, right molar on right; scale = 7 cm, but applicable only to middle distance.

Figure S2. Lateral view of flexed left forelimb of Malolyakhovsky mammoth, outside ice-cave in Kazachie. Anterior toward left. Sole of foot of right hind limb, lying transversely underneath rear of body, appears at right margin of image; more proximal portions of this limb are covered in this view by remnant ice that covered the underside of the specimen.

Figure S3. Lateral view of flexed right forelimb of Malolyakhovsky mammoth, outside ice-cave in Kazachie. Anterior toward right. Skin of neck and underside of mouth has slumped downward in front of right wrist as specimen thawed. Gloved hand at upper right for scale.

Figure S4. Large hematoma (10-cm scale) encountered on right flank of the Malolyakhovsky mammoth. Hematoma was located between two ribs, within the abdominal wall, and is here shown in medial aspect, after exposing it by cutting through the peritoneum and abdominal muscles. Dark reddish-brown mass between gloved hand and photo-scale is coagulated and hemolyzed blood. This hematoma is indicative of some premortem trauma.

Figure S5. Close-up view of hematoma shown in Fig. S4. Dark reddish-brown mass in center of image is coagulated and hemolyzed blood. For scale, refer to Fig. S1.

Figure S6. Resting on an outstretched gloved hand is a portion of the mass of coagulated and hemolyzed blood removed from the hematoma shown in Figs. S4 and S5.

Figure S7. Sagittal section (reconstructed from computed tomography data) through right tibia of the Malolyakhovsky mammoth. Head of yellow arrow points to a small fracture on the posterior

aspect of the tibia. Preserved soft tissue in the vicinity of this defect remains entirely outside the fragment of cortical bone. For scale, see manuscript Fig. 7.

Figure S8. A. Hemolyzed blood observed in the lumen of a blood vessel, where deformed erythrocyte ghosts are visible (numbered square symbols mark six examples). Hematoxylin-eosin stain; red scale bar is 100 µm. B. Cross section of muscle bundles. Deformed myosymplast nuclei (dark purple structures) located under the sarcolemma; section orientation ranges from transverse to oblique relative to long axes of muscle bundles; red scale bar is 100 µm.

Figure S9. Results of phylogenetic analysis showing relationships of microbial taxa cultured and sequenced from samples of the Malolyakhovsky mammoth tissue and intestinal contents. Sizes of yellow circles at some nodes are logarithmically scaled to the number of reads obtained directly for given taxa.

Table S1. Dimensions of tusks of the Malolyakhovsky mammoth.

Measurements	I2 sinister	I2 dexter
Weight	16.6 kg	17.6 kg
Length along outside curve	223 cm	207.5 cm (cracked at base)
Chord length, to far edge	138 cm	139 cm
Chord length, to near edge	132 cm	131 cm
Depth of pulp cavity	11.5 cm	11.9 cm
Tusk circumferences		
@ 10 cm from tip	11.8 cm	16.5 cm
@ 20 cm from tip	15.1 cm	19.0 cm

@ 30 cm from tip	17.4 cm	20.6 cm
@ 40 cm from tip	18.4 cm	22.2 cm
@ 50 cm from tip	19.9 cm	23.5 cm
@ 60 cm from tip	21.6 cm	24.8 cm
@ 70 cm from tip	22.8 cm	26.0 cm
@ 80 cm from tip	24.3 cm	27.2 cm
@ 90 cm from tip	25.5 cm	27.6 cm
@ 100 cm from tip	26.6 cm	28.1 cm
@ 110 cm from tip	27.0 cm	28.2 cm
@ 120 cm from tip	27.5 cm	28.4 cm
@ 130 cm from tip	28.1 cm	29.0 cm
@ 140 cm from tip	29.1 cm	31.0 cm
@ 150 cm from tip	30.0 cm	<32.0 cm
@ 160 cm from tip	31.4 cm	<31.8 cm
@ 170 cm from tip	<31.0 cm	<30.3 cm
@ 180 cm from tip	<29.5 cm	<28.9 cm
@ 190 cm from tip	<28.5 cm	
@ 200 cm from tip	<24.6 cm	

Table S2. Measurements (in mm) of the scapula (following Maschenko, 2002).

Name of measurement	dexter	sinister
Greatest length	>630	>621
Length to middle of glenoid fossa	>590	582

Head greatest width	230	229
Neck width	192	>187

Table S3. Measurements (in mm) of the femur (following Maschenko, 2002).

Name of measurement	dexter	sinister
Patellar facet width	86	91
Block width	157	174
Medio-lateral diameter of distal shaft	187	184
Antero-posterior diameter of distal shaft	200	201
Greatest length	890	>777
Antero-posterior diameter through head	>130	-
Medio-lateral diameter through head	137	-
Shaft length	735	623
Smallest transverse shaft width	116	118
Smallest antero-posterior shaft width	74	71
Distance between lateral condyle and apex of head	895	-

Table S4. Measurements (in mm) of the left tibia (following Maschenko, 2002).

Name of measurement	Measurements in mm
Medio-lateral diameter of the proximal shaft end	181
Antero-posterior diameter of the proximal shaft end	181

Smallest transverse shaft width	78
Smallest antero-posterior shaft width	67
Greatest width of distal articulation	148
Length from distal to proximal articular surfaces	428
Shaft length	406

Table S5. Measurements (in mm) of the left fibula (following Labe, 1999).

Name of measurement	Measurements in mm
Maximum length	486
Medio-lateral diameter of the proximal epiphysis	95
Medio-lateral diameter of the distal epiphysis	57
Antero-posterior diameter of the diaphysis	39
Circumference of the middle of the diaphysis	28

Table S6. Biochemical indices of biological material taken from the Malolyahovsky mammoth carcass.

Biomaterial	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5
Thawing time	03/11/14 19.30 h.	03/11/14 20 h.	03/11/14 21 h.	03/12/14 43 h.	03/13/14 67 h.
Indices	Substrates				
Iron, mmol/L	546.70	-	535.50	2510.80	2.70

Urea, mmol/L	38.44	102.57	39.37	116.35	7.37
Creatinine, mmol/L	147.20	76.0	141.80	274.40	5.90
		Enzymes			
LDH – lactate dehydrogenase mmol/L	8.0	2.0	5.0	-	2.0
Gamma glutamyltransferase mmol/L	7.0	4.0	5.0	3.0	5.0
AP - alkaline phosphatase mmol/L	9.0	7.0	7.0	5.0	-
CPK - Creatinphosphokinase (total) mmol/L	11.3	5.4	11.5	-	4.2
Alpha-amylase mmol/L	3.2	7.6	3.8	-	-
ALT - alanine aminotransferase mmol/L	14.0	25.0	11.0	-	14.0
AAT - aspartate aminotransferase mmol/L.	-	45.0	25.0	269.0	-
Lipase mmol/L	1.44	1.84	2.43	3.05	-