

Supplementary Information

Substitutions in woolly mammoth hemoglobin confer biochemical properties adaptive for cold tolerance

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Supplementary materials include:

Supplementary Figures S1–S12

Supplementary Tables S1–S4

Supplementary Note

SUPPLEMENTARY NOTE

Characterization of Asian/African elephant α -like and β -like globin genes

The α - and β -globin gene families of eutherian mammals have undergone numerous (and in many cases independent) tandem and *en bloc* duplications, interparalog gene conversions, silencing and deletion events since their divergence from metatherians^{1,2}. Unfortunately, inconsistent use of the original Greek alphabet nomenclature system adopted for the loci of these gene clusters fails to reflect their complex evolutionary history, thus making it difficult to not only distinguish paralogous from orthologous sequences among species, but also silenced from functional genes³. We thus adopted the three-letter nomenclature system of Aguileta *et al.*³ to best reflect the evolutionary origin of genes amplified/sequenced in this study. Within each cluster, tandemly duplicated genes are designated with the symbol -T followed by a number that corresponds to their 5' to 3' linkage order, while pseudogenes are indicated by a lowercase "ps". We classified globin-like loci as transcriptionally silent if they possessed a premature stop codon or lacked any of the following important characteristics of functional globin genes^{4,5}: a) a 3 exon/2 intron structure, b) transcriptional control motifs common to the 5' flanking region of α - or β -globin cluster genes, c) an 'ATG' initiation codon, d) 5'/3' GT-AG splice junction signals flanking each intron, and e) an intact poly-A signal in the 3' transcribed flanking region.

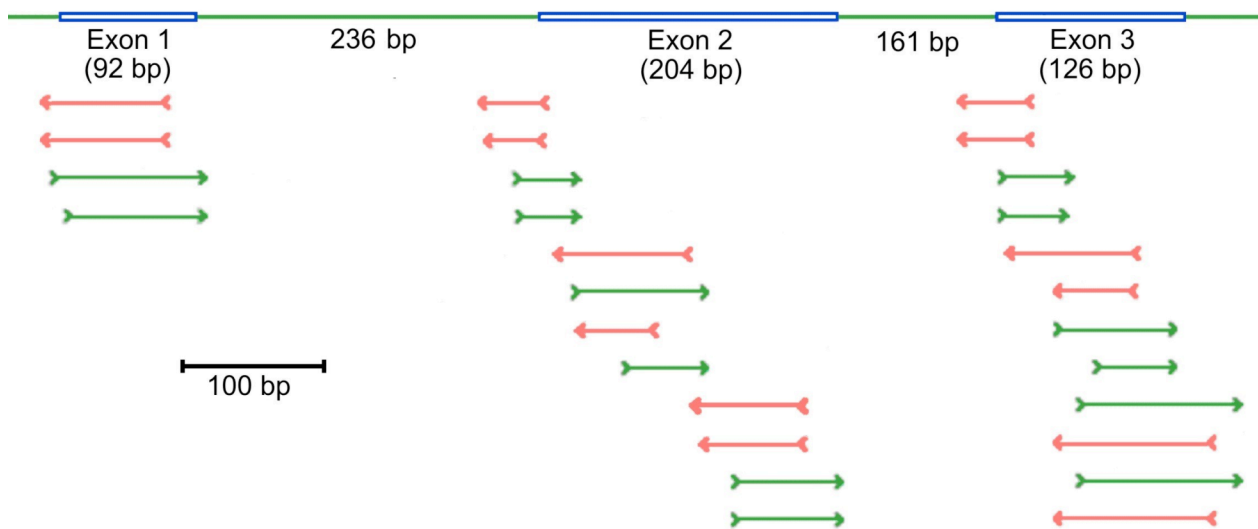
Functional mammalian α -like and β -like globin genes are composed of three coding regions (normally comprising 92, 205 and 126 bp, and 89, 223 and 126 bp, respectively) separated by two intervening sequences of variable length, and flanked by transcriptionally important upstream and downstream noncoding regions^{4,5} (see also Supplementary Figs. 2 and 3). We amplified complete, orthologous α -like and β -like globin gene regions from the DNA of Asian (1010 and 2119 bp, respectively) and African (2335 and 1744 bp, respectively) elephants. We then utilized working draft BAC sequences of the African elephant to determine the identity of these two adult-expressed genes. Scaffolds spanning the entire α -globin cluster were downloaded from GenBank (GB AC158446.2, AC160597.2, AC175811.2) and dotmatcher (EMBOSS) software was used to visualize regions of high homology with the three exons of the transcribed African α -like gene product we obtained. The dotplot representation identified six distinct α -like globin loci within the GenBank *Loxodonta* contigs. BlastN (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) and percent identity plots against known paralogs/orthologs were used to characterize the six α -like genes as follows: 5'–*HBZ-T1*, *HBZ-T2*, *HBK*, *HBA-T1ps*, *HBA-T2*, *HBQ*–3'. This result differs slightly from the phylogenetically deduced 5'–*HBZ-T1*, *HBZ-T2*, *HBA-T1ps*, *HBA-T2*, *HBQ*–3' cluster arrangement recently published by Hoffmann *et al.*². Exon and mRNA sequences of the African elephant α -like globin product we sequenced precisely matched the *Loxodonta HBA-T2* locus.

Two non-overlapping BAC sequences from GenBank (AC167954.3, jxy; AC169166.2, ktd) and the super_3994 contig of the Broad Institute 2× elephant assembly loxAfr1 (May 2005;

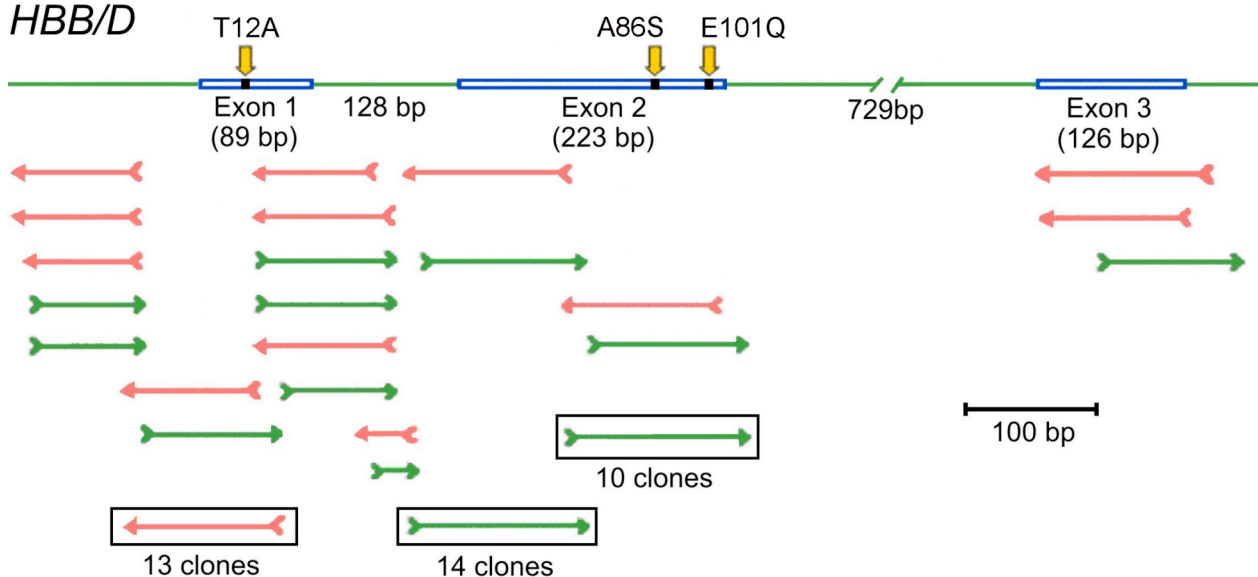
<http://genome.ucsc.edu/>), were also downloaded and examined using the procedures and criteria outlined above. Dotplot comparisons of the *Loxodonta* GenBank contigs against the exon sequences of the transcribed African 'β-like' gene product we obtained revealed five β-like globin loci, while four globin genes were detected in the super_3994 strand, which we attribute to a sequence gap in this later contig. BlastN, dotplot and MATGAT analyses were used to designate these genes as: 5'–*HBE*, *HBG*, *HBDps*, *HBB/D*, *HBBps*–3', which is identical to the phylogenetically deduced arrangement of Opazo *et al.*⁶. Exon and mRNA sequences of our African elephant β-like globin product precisely matched the *HBB/D* locus. Thus, the single adult-expressed hemoglobin component of elephantids is encoded by the *HBA-T2* (α-chain) and *HBB/D* (β/δ-chain) loci.

Supplementary Figure 1. Schematic of the redundant overlapping sequencing strategy employed in this study to obtain the complete coding sequence of the *HBA-T2* (α) and *HBB/D* (β/δ) globin genes of woolly mammoth specimen SP1349. The lengths of the three coding exons and intervening sequences for each gene are given. Red and green arrows denote the direction each PCR product was directly sequenced. Cloned regions are outlined, with the number of clones sequenced indicated below each box. The locations of the three mammoth-specific amino-acid substitutions are indicated by arrows.

HBA-T2



HBB/D



Supplementary Figure 2. DNA sequences of African elephant (*Loxodonta africana*), Asian elephant (*Elephas maximus*) and woolly mammoth (*Mammuthus primigenius*) HBA-T2 globin genes amplified in this study. Consensus sequences are presented for the two extant species while the 28 separate direct sequencing (F=forward, R=reverse) reactions from woolly mammoth specimen SP1349 are given. The transcribed mRNA sequence is identified by upper case letters, while intervening and nontranscribed flanking sequences are shown in lower case. Initiation (ATG) and stop (TGA) codons are underlined and bold. The 5' mRNA cap site ('CAP') and 3' poly-adenylation signal ('AATAAA') are underlined. Synonymous and nonsynonymous nucleotide changes are denoted by red and bold red characters, respectively. Within each coding block, the translated amino-acid sequences of the expressed woolly mammoth α -globin chain are listed below the corresponding base triplets.

```

-40      -30      -20      -10      1
|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .
Loxodonta africana  cACGCTTCTGATCTCCACACAGACTCAGAAACAACCCACCATGGTGCT
Elephas maximus   cACGCTTCTGATCTCCACACAGACTCAGAAACAACCCACCATGGTGCT
SP1349_Alpha2_R1.3_B05 -----AGAAACAACCCACCATGGTGCT
SP1349_Alpha2_R1.3_C01 -----AGAAACAACCCACCATGGTGCT
SP1349_Alpha2_F1.1_B01 -----AACCCACCATGGTGCT
SP1349_Alpha2_F1.1_A05 -----GGTGCT
'          CAP'

10      20      30      40      50
.|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|.
Loxodonta africana  GTCTGATAACGACAAGACCAACGTC AAGGCCACCTGGAGCAAGGTTGG
Elephas maximus   GTCTGATAAGGACAAGACCAACGTC AAGGCCACCTGGAGCAAGGTTGG
SP1349_Alpha2_R1.3_B05 GTCTGATAACGACAAGACCAACGTC AAGGCCACCTGGAGCAAGGTTGG
SP1349_Alpha2_R1.3_C01 GTCTGATAACGACAAGACCAACGTC AAGGCCACCTGGAGCAAGGTTGG
SP1349_Alpha2_F1.1_B01 GTCTGATAACGACAAGACCAACGTC AAGGCCACCTGGAGCAAGGTTGG
SP1349_Alpha2_F1.1_A05 GTCTGATAACGACAAGACCAACGTC AAGGCCACCTGGAGCAAGGTTGG
uSerAspAsnAspLysThrAsnValLysAlaThrTrpSerLysValGl

60      70      80      90
...|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|IVS1→
Loxodonta africana  CGACCACGCTTCGGATTATGTCGCCGAGGCCCTGGAGAGgtgaggatc
Elephas maximus   CGACCACGCTTCGGATTATGTCGCCGAGGCCCTGGAGAGgtgaggatc
SP1349_Alpha2_R1.3_B05 CGACCACGCTTCGGAT-----
SP1349_Alpha2_R1.3_C01 CGACCACGCTTCGGAT-----
SP1349_Alpha2_F1.1_B01 CGACCACGCTTCGGATTATGTCGCCGAGGCCCTGGAGAGgtga----
SP1349_Alpha2_F1.1_A05 CGACCACGCTTCGGATTATGTCGCCGAGGCCCTGGAGAGgtga----
yAspHisAlaSerAspTyrValAlaGluAlaLeuGluAr

Loxodonta africana  ccctgtcccctacttccacgcacctggggtcaacaccaccccactgc
Elephas maximus   ccctgtcccctacttccacgcacctggggtcaacaccaccccactgc

Loxodonta africana  ccacgcgtgggctacaaccaggccggtgtctaggcccaaggccccga
Elephas maximus   ccacgcgtgggctacaaccaggccggtgtctaggcccaaggccccga

Loxodonta africana  tgcccagaccaccgacctcccgccaggggagcccggccctcctatccg
Elephas maximus   tgcccagaccaccgacctcccgccaggggagcccggccctcctatccg

Loxodonta africana  ccctccctcagaggctgtgtgtgtgtctcctgttcccctaccctacc
Elephas maximus   ccctccctcagaggctgtgtgtgctctcctgtctcccctaccctacc
SP1349_Alpha2_R2.2N_B02 -----cc
SP1349_Alpha2_R2.2N_F02 -----cc

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                                                    96           100
                                                    .....|.....|...
Loxodonta africana          ctccaccaccgctcacctgccacttctccacgcagGATGTTCTTCTCC
Elephas maximus          ctccaccaccgctcacctgccacttctccacgcagGATGTTCTTCTCC
SP1349_Alpha2_R2.2N_B02    ctccaccaccgctcacctgccacttctccacgcagGATGT-----
SP1349_Alpha2_R2.2N_F02    ctccaccaccgctcacctgccacttctccacgcagGA-----
SP1349_Alpha2_F2N_A02      -----ctgccacttctccacgcagGATGTTCTTCTCC
SP1349_Alpha2_F2N_E02      -----gccacttctccacgcagGATGTTCTTCTCC
SP1349_Alpha2_R2.3N_E01    -----CC
                                                    gMetPhePheSer

          110           120           130           140           150
          .|.....|.....|.....|.....|.....|.....|.....|.....|.....|..
Loxodonta africana          TTTCCACCACCAAGACCTACTTTCCTCACTTCGACCTGGCCATGGC
Elephas maximus          TTTCCACCACCAAGACCTACTTTCCTCACTTCGACCTGAGCCATGGC
SP1349_Alpha2_F2N_A02      TTTCCACCACC-----
SP1349_Alpha2_F2N_E02      TTTCCACCACC-----
SP1349_Alpha2_R2.3N_E01    TTTCCACCACCAAGACCTACTTTCCTCACTTCGACCTGAGCCATGGC
SP1349_Alpha2_F2.3_D01     -----CCAAGACCTACTTTCCTCACTTCGACCTGAGCCATGGC
SP1349_Alpha2_R2.3N_H05    -----GACCTACTTTCCTCACTTCGACCTGAGCCATGGC
SP1349_Alpha2_F2.3N_G05    -----GGC
PheProThrThrLysThrTyrPheProHisPheAspLeuSerHisGly

          160           170           180           190           200
          ...|.....|.....|.....|.....|.....|.....|.....|.....|.....
Loxodonta africana          TCTGGCCAGGTCAAGGCACATGGCAAGAAGGTGGGGGAAGCACTGACC
Elephas maximus          TCTGGCCAGGTCAAGGGACATGGCAAGAAGGTGGGGGAAGCACTGACC
SP1349_Alpha2_R2.3N_E01    TCTGGCCAGGTCAAGGGACATGGCAAGAAGGTGGGGGAAGCAC-----
SP1349_Alpha2_R2.3_D01     TCTGGCCAGGTCAAGGGACATGGCAAGAAGGTGGGGGAAGCACTGACC
SP1349_Alpha2_R2.3N_H05    TCTGGCCAGGTCAAGGGAC-----
SP1349_Alpha2_F2.3N_G05    TCTGGCCAGGTCAAGGGACATGGCAAGAAGGTGGGGGAAGCACTGACC
SP1349_Alpha2_R2.4N_D02    -----CC
SerGlyGlnValLysGlyHisGlyLysLysValGlyGluAlaLeuThr

          210           220           230           240           250
          |.....|.....|.....|.....|.....|.....|.....|.....|.....|..
Loxodonta africana          CAAGCTGTTGGCCACCTGGATGACCTGCCTAGCGCCCTGTCTGCACTC
Elephas maximus          CAAGCTGTTGGCCACCTGGATGACCTGCCTAGCGCCCTGTCTGCACTC
SP1349_Alpha2_F2.3_D01    CAAGCT-----
SP1349_Alpha2_R2.3N_G05    CAAGCT-----
SP1349_Alpha2_R2.4N_D02    CAAGCTGTTGGCCACCTGGATGAYCTGCCTAGCGCCCTGTCTGCACTC
SP1349_Alpha2_R2.4N_H02    ---GCTGTTGGCCACCTGGATGACCTGCCTAGCGCCCTGTCTGCACTC
SP1349_Alpha2_F2.4_C02     -----CTGCCTAGCGCCCTGTCTGCACTC
SP1349_Alpha2_F2.4N_G02    -----TGCCCTAGCGCCCTGTCTGCACTC
GlnAlaValGlyHisLeuAspAspLeuProSerAlaLeuSerAlaLeu

          260           270           280           290           300
          ..|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Loxodonta africana          AGCGACCTGCACGCTCACAAGCTGAGGGTGGACCCGTGCAACTTCAAG
Elephas maximus          AGCGACCTGCACGCTCACAAGCTGAGGGTGGACCCGTGCAACTTCAAG
SP1349_Alpha2_R2.4N_D02    AGCGACCTGCACGCTCACAAGCTGAGG-----
SP1349_Alpha2_R2.4N_H02    AGCGACCTGCACGCTCACAAGCTGAGG-----
SP1349_Alpha2_F2.4_C02     AGCGACCTGCACGCTCACAAGCTGAGGGTGGACCCGTGCAACTTCAAG
SP1349_Alpha2_F2.4N_G02    AGCGACCTGCACGCTCACAAGCTGAGGGTGGACCCGTGCAACTTCAAG
SerAspLeuHisAlaHisLysLeuArgValAspProValAsnPheLys

IVS2→
Loxodonta africana          gtgaggagagggaaagacctggaaggtcggggcagcaggccttctctgg

```

Elephas maximus
 SP1349_Alpha2_F2.4_C02
 SP1349_Alpha2_F2.4N_G02

gtgaggagaggggaagacctggaaggtcggggcagcaggccttcctgg
 gtga-----
 gtga-----

Loxodonta africana
Elephas maximus
 SP1349_Alpha2_R3.2_C02
 SP1349_Alpha2_R3.2N_E02

cagggccgagatctgcaaaggtgagcgttcacctaacagcccccttgtc
 cagggccgagatctgcaaaggtgagggttccctaacagcccccttgtc
 -----cccccttgtc
 -----cccccttgtc

Loxodonta africana
Elephas maximus
 SP1349_Alpha2_R3.2_C02
 SP1349_Alpha2_R3.2N_E02
 SP1349_Alpha2_F3N_B02
 SP1349_Alpha2_F3N_D02
 SP1349_Alpha2_R3N_H02

301 310 320 330
|....|....|....|....|....|....|
 ggccccctctctccacagCTCCTGAGCCACTGCCTGCTGGTGACTCTGA
 gccccctctctccacagCTCCTGAGCCACTGCCTGCTGGTGACTCTAA
 gccccctctctccacagCTCCTGAGCCACTGCCTGCTGG-----
 gccccctctctccacagCTCCTGAGCCACTGCCTGCTGG-----
 -----CTCCTGAGCCACTGCCTGCTGGTGACTCTGA
 -----CTCCTGAGCCACTGCCTGCTGGTGACTCTGA
 -----AGCCACTGCCTGCTGGTGACTCTGA
LeuLeuSerHisCysLeuLeuValThrLeuS

Loxodonta africana
Elephas maximus
 SP1349_Alpha2_F3N_B02
 SP1349_Alpha2_F3N_D02
 SP1349_Alpha2_R3N_H02
 SP1349_Alpha2_F3.1N_G02
 SP1349_Alpha2_R3N_D03
 SP1349_Alpha2_F3.2_C03
 SP1349_Alpha2_F3.2_F01
 SP1349_Alpha2_R3_G01
 SP1349_Alpha2_F3.2_H01
 SP1349_Alpha2_R3_B02

340 350 360 370
 ...|...|...|...|...|...|...|...|...|...|...
 GCAGCCACCAACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
 GCAGCCACCAACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
 GCAGCCACCAACCCACGGA-----
 GCAGCCACCAACCCACGGA-----
 GCAGCCACCAACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
 ---GCCACCAACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
 -----AACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
 -----CATGCCTCCCTGG
 -----AGTTCACCCCTGAGGTCCATGCCTCCCTGG
 -----AACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
 -----TTCACCCCTGAGGTCCATGCCTCCCTGG
 -----AACCCACGGAGTTCACCCCTGAGGTCCATGCCTCCCTGG
erSerHisGlnProThrGluPheThrProGluValHisAlaSerLeuA

Loxodonta africana
Elephas maximus
 SP1349_Alpha2_F3.1N_G02
 SP1349_Alpha2_R3N_H02
 SP1349_Alpha2_R3N_D03
 SP1349_Alpha2_F3.2_C03
 SP1349_Alpha2_F3.2_F01
 SP1349_Alpha2_R3_G01
 SP1349_Alpha2_F3.2_H01
 SP1349_Alpha2_R3_B02

380 390 400 410 420
 |...|...|...|...|...|...|...|...|...|...|...|
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAATATCGT
 ACAAGTTCCTTAGCAACGTGAGCACCCTGACCTCCAAATATCGT
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAAT-----
 ACAAGTTCCTCAGCAAC-----
 ACAAGTTCCTCAGCAAC-----
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAAT-----
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAATATCGT
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAATATCGT
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAATATCGT
 ACAAGTTCCTCAGCAACGTGAGCACCCTGACCTCCAAATATCGT
spLysPheLeuSerAsnValSerThrValLeuThrSerLysTyrArg

Loxodonta africana
Elephas maximus
 SP1349_Alpha2_F3.2_F01

+1 +10 +20 +30 +40
 ...|...|...|...|...|...|...|...|...|...|...|
TAAGCTGGACCCCAGGAGAGGTGCCACTGGCCTTCCTCCCCCTCCCTGC
TAAGCTGGACCCCAGGAGAGGTGCCACTGGCCTTCCTCCCCCTCCCTGC
TAAGCTGGACCCCAGGAGAGG-----

SP1349_Alpha2_R3_G01
 SP1349_Alpha2_F3.2_H01
 SP1349_Alpha2_R3_B02

TAAGCTGGACCCAGGAGAGG-----
TAAGCTGGACCCAGGAGAGG-----
TAAGCTGGACCCAGGAGAGG-----

Loxodonta africana
Elephas maximus

+50 +60 +70 +80 +90
 |...|...|...|...|...|...|...|...|...|..
 ATGTGCCACCAAGTCTTTGAATAAACTCTGAGTGGGCAGCagcctggt
 ATGTGCCACCAAGTCTTTGAATAAACTCTGAGTGGGCAGCagcctggt
 'AATAAA'

Supplementary Figure 3. DNA sequences of African elephant (*Loxodonta africana*), Asian elephant (*Elephas maximus*) and woolly mammoth (*Mammuthus primigenius*) *HBB/D* globin genes amplified in this study. Consensus sequences are presented for the two extant species while the 21 separate direct sequencing reactions (F=forward, R=reverse) from woolly mammoth specimen SP1349 are given. The transcribed mRNA sequence is identified by upper case letters, while intervening and nontranscribed flanking sequences are shown in lower case. Initiation (ATG) and stop (TGA) codons are underlined and bold. The 5' upstream transcriptional control motifs necessary for transcription of a functional product ('CACCC', 'CCAAT', 'ATA'), the mRNA cap site ('CAP') and the 3' poly-adenylation signal ('AATAAA') are underlined. Synonymous and nonsynonymous nucleotide changes are denoted by red and bold red characters, respectively. Within each coding block, the translated amino-acid sequences of the expressed woolly mammoth β/δ -globin chain are listed below the corresponding base triplets.

```

-160      -150      -140      -130      -120
...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|.
Loxodonta africana   gcctcaccctgcagaaccaaccctggccttggccaatctg:tcacaa
Elephas maximus    gcctcaccctgcagaaccaaccctggccttggccaatctgctcacia
SP1349_Delta_Upstr_R_E01  ---taccctgcagaaccaaccctggccttggccaatctgctcacia
SP1349_Delta_Upstr_R_B02  ---taccctgcagaaccaaccctggccttggccaatctgctcacia
SP1349_Delta_Upstr_R_G03  -----agaaccacaccctggccttggccaatctgctcacia
SP1349_Delta_Upstr_F_F03  -----cacaccctggccttggccaatctgctcacia
SP1349_Delta_Upstr_F_A02  -----cacaccctggccttggccaatctgctcacia
                        'CACCC'      'CACCC'      'CCAAT'

-110      -100      -90      -80      -70
...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|.
Loxodonta africana   gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
Elephas maximus    gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
SP1349_Delta_Upstr_R_E01  gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
SP1349_Delta_Upstr_R_B02  gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
SP1349_Delta_Upstr_R_G03  gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
SP1349_Delta_Upstr_F_F03  gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
SP1349_Delta_Upstr_F_A02  gagcaaaaagggcaggaccagggttgggcatataaggaagagtagtgc
                        'ATA'

-60      -50      -40      -30      -20
|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|.
Loxodonta africana   cagctgctgtttACACTCACTTCTGACACAACTGTGTTGACTAGCAAC
Elephas maximus    cagctgctgtttACACTCACTTCTGACACAACTGTGTTGACTAGCAAC
SP1349_Delta_Upstr_R_E01  cagctgct-----
SP1349_Delta_Upstr_R_B02  cagctgctg-----
SP1349_Delta_Upstr_R_G03  cagctgct-----
SP1349_Delta_Upstr_F_F03  cagctgctgttt-----
SP1349_Delta_Upstr_F_A02  cagctgctgttt-----
SP1349_Delta_R1N_F02     cagctgctgtttACACTCACTTCTGACACAACTGTGTTGACTAGCAAC
SP1349_Delta_F1N_E02     -----TTCTGACACAACTGTGTTGACTAGCAAC
                        'CAP'

-10      1      10      20      30
.|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ..
Loxodonta africana   TACCCAATCAGACACCATGGTGAATCTGACTGCTGCTGAGAAAGACACA
Elephas maximus    TACCCAATCAGACACCATGGTGAATCTGACTGCTGCTGAGAAAGACACA
SP1349_Delta_R1N_F02  TACCCAATCAGACACCATGGTGAATCTGACTGCTGCTGAGAAAGACACA
SP1349_Delta_F1N_E02  TACCCAATCAGACACCATGGTGAATCTGACTGCTGCTGAGAAAGACACA
                        ValAsnLeuThrAlaAlaGluLysThrG1

Mammoth A G (T12A) change
↓ 40      50      60      70      80
..|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|.

```

Loxodonta africana
Elephas maximus
 SP1349_Delta_R1N_F02
 SP1349_Delta_F1N_E02
 SP1349_Delta_R2N_H02
 SP1349_Delta_F2N_G02
 SP1349_Delta_R2N_B01
 SP1349_Delta_F2N_A01
 SP1349_Delta_R2N_D01
 SP1349_Delta_F2N_C01

AGTCACCAACCTGTGGGGCAAGGTGAATGTGAAAGAGCTTGGTGGTGA
 AGTCACCAACCTGTGGGGCAAGGTGAATGTGAAAGAGCTTGGTGGTGA
 AGTC**G**CCAACCTG-----
 AGTC**G**CCAACCTGTGGGGCAAGGTGAATGT-----
 -----GGCAAGGTGAATGTGAAAGAGCTTGGTGGTGA
 -----CTTGGTGGTGA
 -----GGCAAGGTGAATGTGAAAGAGCTTGGTGGTGA
 -----GGTGAATGTGAAAGAGCTTGGTGGTGA
 -----CAAGGTGAATGTGAAAGAGCTTGGTGGTGA
 -----GGTGAATGTGAAAGAGCTTGGTGGTGA
nValAlaAsnLeuTrpGlyLysValAsnValLysGluLeuGlyGlyGl

90

....|....|..IVS1→

Loxodonta africana
Elephas maximus
 SP1349_Delta_R2N_H02
 SP1349_Delta_F2N_G02
 SP1349_Delta_R2N_B01
 SP1349_Delta_F2N_A01
 SP1349_Delta_R2N_D01
 SP1349_Delta_F2N_C01

GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
 GGCCCTGAGCAGgtttctatctaggttgcaaggtagacttaaggaggg
uAlaLeuSerAr

Loxodonta africana
Elephas maximus
 SP1349_Delta_R2N_H02
 SP1349_Delta_F2N_G02
 SP1349_Delta_R2N_B01
 SP1349_Delta_F2N_A01
 SP1349_Delta_R2N_D01
 SP1349_Delta_R2_EO2
 SP1349_Delta_F2.2_D02
 SP1349_Delta_R3N_E03

ttgagtggggctgggcatgtggagacagaacagtctcccagtttctga
 ttgagtggggctgggcatgtggagacagaacagtctcccagtttctga
 ttgagtggg-----
 ttgagtggggctgggcatgtggagacagaacag-----
 ttgagtggggctgggcatgtggagacagaacag-----
 ttgagtggggctgggcatgtggagacagaacag-----
 ttgagtggggctgggcatgtggagacag-----
 ttgagtggggctgggcatgtggagacagaacag-----
 -----gggctgggcatgtggagacagaacagtctcccagtttctga
 -----atgtggagacagaacagtctcccagtttctga
 -----a

93

..|. |.

Loxodonta africana
Elephas maximus
 SP1349_Delta_R2_EO2
 SP1349_Delta_F2.2_D02
 SP1349_Delta_R3N_E03
 SP1349_Delta_F3N_D03

caggcactgacttcctctgcaccctgtggtgctttcaccttcagGCTG
 caggcactgacttcctctgcaccctgtggtgctttcaccttcagGCTG
 ca-----
 cag-----
 caggcactgacttcctctgcaccctgtggtgctttcaccttcagGCTG
 -----ctgtggtgctttcaccttcagGCTG
gLeu

100 110 120 130 140

...|...|...|...|...|...|...|...|...|...|...

Loxodonta africana
Elephas maximus
 SP1349_Delta_R3N_E03
 SP1349_Delta_F3N_D03

CTGGTGGTCTACCCATGGACCCGGAGGTTCTTTGAACACTTTGGGGAC
 CTGGTGGTCTACCCATGGACCCGGAGGTTCTTTGAACACTTTGGGGAC
 CTGGTGGTCTACCCATGGACCCGGAGGTTCTTTGAACACTTTGGGGAC
 CTGGTGGTCTACCCATGGACCCGGAGGTTCTTTGAACACTTTGGGGAC
LeuValValTyrProTrpThrArgArgPhePheGluHisPheGlyAsp

150 160 170 180 190

|...|...|...|...|...|...|...|...|...|..

Loxodonta africana
Elephas maximus
 SP1349_Delta_R3N_E03
 SP1349_Delta_F3N_D03

CTGTCCACTGCTGA**A**GCTGTCCTGCACAACGCTAAAGTGCTGGCCCAT
 CTGTCCACTGCTGACGCTGTCCTGCACAACGCTAAAGTGCTGGCCCAT
 CTGTCCACTGCTGACGCTGTCCTGCACAACG-----
 CTGTCCACTGCTGACGCTGTCCTGCACAACGCTAAAGTGCTGGCCCAT

SP1349_Delta_R4_B01

-----GTGCTGGCCCAT
LeuSerThrAlaAspAlaValLeuHisAsnAlaLysValLeuAlaHis

Loxodonta africana
Elephas maximus
 SP1349_Delta_F3N_D03
 SP1349_Delta_R4_B01
 SP1349_Delta_F4.1N_A01

 200 210 220 230 240
 ..|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|
 GGCGAGAAAGTGTGACCTCCTTTGGTGAGGGCTGAAGCACCTGGAC
 GGCGAGAAAGTGTGACCTCCTTTGGTGAGGGCCTGAAGCACCTGGAC
 GG-----
 GGCGAGAAAGTGTGACCTCCTTTGGTGAGGGCCTGAAGCACCTGGAC
 -----AGTGTGACCTCCTTTGGTGAGGGCCTGAAGCACCTGGAC
GlyGluLysValLeuThrSerPheGlyGluGlyLeuLysHisLeuAsp

Loxodonta africana
Elephas maximus
 SP1349_Delta_R4_B01
 SP1349_Delta_F4.1N_A01

 Mammoth G T (A86S) change
 ↓
 250 260 270 280
|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|
 AACCTCAAGGGCACCTTTGCCGATCTGAGCGAGCTGCACCTGTGACAAG
 AACCTCAAGGGCACCTTTGCCGATCTGAGCGAGCTGCACCTGTGACAAG
 AACCTCAAGGGCACCTTT**T**CCGATCTGAGCGAGCTGCACCTGTGACAAG
 AACCTCAAGGGCACCTTT**T**CCGATCTGAGCGAGCTGCACCTGTGACAAG
AsnLeuLysGlyThrPheSerAspLeuSerGluLeuHisCysAspLys

Loxodonta africana
Elephas maximus
 SP1349_Delta_R4_B01
 SP1349_Delta_F4.1N_A01

 Mammoth G C (E101Q) change
 ↓
 290 30 310
 .|. ...|. ...|. ...|. ...|. ...| IVS2→
 CTGCACGTGGATCCTGAGAATTTTCAGGgtgagtctaggagacattcta
 CTGCACGTGGATCCTGAGAATTTTCAGGgtgagtctaggagacattcta
 CTGCACGTGGATCCT**C**AGA-----
 CTGCACGTGGATCCT**C**AGAATTTTCAGGgtgagtctaggagacattcta
LeuHisValAspProGlnAsnPheArg

Loxodonta africana
Elephas maximus

ttttttcttttccactttgtagtctttcactgtcattatthttgcttatt
 ttttttcttttccactttgtagtctttcactgtcattatthttgcttatt

Loxodonta africana
Elephas maximus

tgaatttcctctgtatctctttttactcgactatgthttcatcatttag
 tgaatttcctctgtatctctttttactcgactatgthttcatcatttag

Loxodonta africana
Elephas maximus

tgctttttcaacttataccattttgtattactttttctttcaatattct
 tg**t**ttttttcaacttataccattttgtattactttttctttcaatattct

Loxodonta africana
Elephas maximus

tccttttttctgactcacattcttgctttatatcatgctctttattt
 tccttttttctgactcacattcttgctttatatcatgctctttattt

Loxodonta africana
Elephas maximus

aatttcctacgthttttgctcttgctctccctttctcctagtttccttc
 aatttcct**g**cgthttttgctcttgctctccctttctccta**a**tttccttc

Loxodonta africana
Elephas maximus

cctctgaacagtaccagaatgtgcataccacctctcatccactattt
 cctctgaacagtacc**a**aatgtgcataccacctctc**g**tccactattt

Loxodonta africana
Elephas maximus

ctgcaactggggcaaatccccaccctcctccatagagggttgaaag
 ctgcaactggggcaaatccccaccctcctccatagagggttgaaag

Loxodonta africana
Elephas maximus

gactgaatcaaagaggagaggatcatcgtgctgtttctagagctgtgga
 gactgaatcaaagaggagaggatcat**g**gtgctgtttctagag**a**tgtga

Loxodonta africana
Elephas maximus

ttcattttcagacttgaaggataacttgaataatataaaatcaggagta
 ttcattttcagacttgaaggataacttgaataatataaaatcaggagta

Loxodonta africana
Elephas maximus

aatggagaggaaagttagtctgagaatgaaagatcagaaggtcata
 aatggagaggaaagttagtctgagaatgaaagatcagaaggtcata

Loxodonta africana gacgagatggggagcagaagttactaagaaactgaccattgtggctat
Elephas maximus gacgagatggggagcagaagttactaagaaactgaccattgtggctat

Loxodonta africana aattaatcacttaattagttaattaatgatgtttgttatttattcacgt
Elephas maximus aattaatcacttaattagttaattaatgatgtttgttatttattcacgt

Loxodonta africana ttttcattttgggtgggagtaaatttgggctagtgtgtgggcaacataa
Elephas maximus ttttcattttgggtgggagtaaatttgggctagtgtgtgggcaacataa

Loxodonta africana atgggtttcaccccattgtctcagaggccaagctggattgctttgtta
Elephas maximus atgggtttcaccccattgtctcagaggccaagctggattgctttgtta

320

Loxodonta africana|.....|..
Elephas maximus accatgtctgtgatgtatctacctcttccccatagCTCCTGGGCAAT
 SP1349_Delta_R5N_D02 accatgtctgtgatgtatctacctcttccccatagCTCCTGGGCAAT
 SP1349_Delta_R5N_G03 -----CTCCTGGGCAAT
 -----CTCCTGGGCAAT
LeuLeuGlyAsn

330 340 350 360 370

Loxodonta africana ..|...|...|...|...|...|...|...|...|...|
Elephas maximus GTGCTGGTGATTGCCTGGCCCGCCACTTTGGCAAGGAATTCACCCCA
 SP1349_Delta_R5N_D02 GTGCTGGTGATTGCCTGGCCCGCCACTTTGGCAAGGAATTCACCCCA
 SP1349_Delta_R5N_G03 GTGCTGGTGATTGCCTGGCCCGCCACTTTGGCAAGGAATTCACCCCA
 SP1349_Delta_F5_F03 GTGCTGGTGATTGCCTGGCCCGCCACTTTGGCAAGGAATTCACCCCA
 -----CCCGCCACTTTGGCAAGGAATTCACCCCA
ValLeuValIleValLeuAlaArgHisPheGlyLysGluPheThrPro

380 390 400 410 420

Loxodonta africana|.....|.....|.....|.....|.....|.....|.....|.....|..
Elephas maximus GATGTT CAGGCTGCCTATGAGAAGGTTGTGGCTGGTGTGGCGAATGCC
 SP1349_Delta_R5N_D02 GATGTT CAGGCTGCCTATGAGAAGGTTGTGGCTGGTGTGGCGAATGCC
 SP1349_Delta_R5N_G03 GATGTT CAGGCTGCCTATGAGAAGGTTGTGGCTGGTGTGGCGAATGCC
 SP1349_Delta_F5_F03 GATGTT CAGGCTGCCTATGAGAAGGTTGTGGCTGGTGTGGCGAATGCC
AspValGlnAlaAlaTyrGluLysValValAlaGlyValAlaAsnAla

430 440 +1 +10 +20

Loxodonta africana .|...|...|...|...|...|...|...|...|...|..
Elephas maximus CTGGCTCACAAATACCAC**TGAG**ATCCTGGCCTGTTCCCTGGTATCCATC
 SP1349_Delta_R5N_D02 CTGGCTCACAAATACCAC**TGAG**ATCCTGGCCTGTTCCCTGGTATCCATC
 SP1349_Delta_R5N_G03 CTGGCTCACAAATACCAC**TGAG**ATCCTGGCCTGTTCC-----
 SP1349_Delta_F5_F03 CTGGCTCACAAATACCAC**TGAG**ATC-----
LeuAlaHisLysTyrHis

+30 +40 +50 +60 +70

Loxodonta africana ..|...|...|...|...|...|...|...|...|...|..
Elephas maximus GGAAGCCCCATTTCCCGAGATGCTATCTCTGAATTTGGGAAAATAATG
 SP1349_Delta_F5_F03 GGAAGCCCCATTTCCCGAGATGCTATCTCTGAATTTGGGAAAATAATG
 GGAAGCCCCA-----

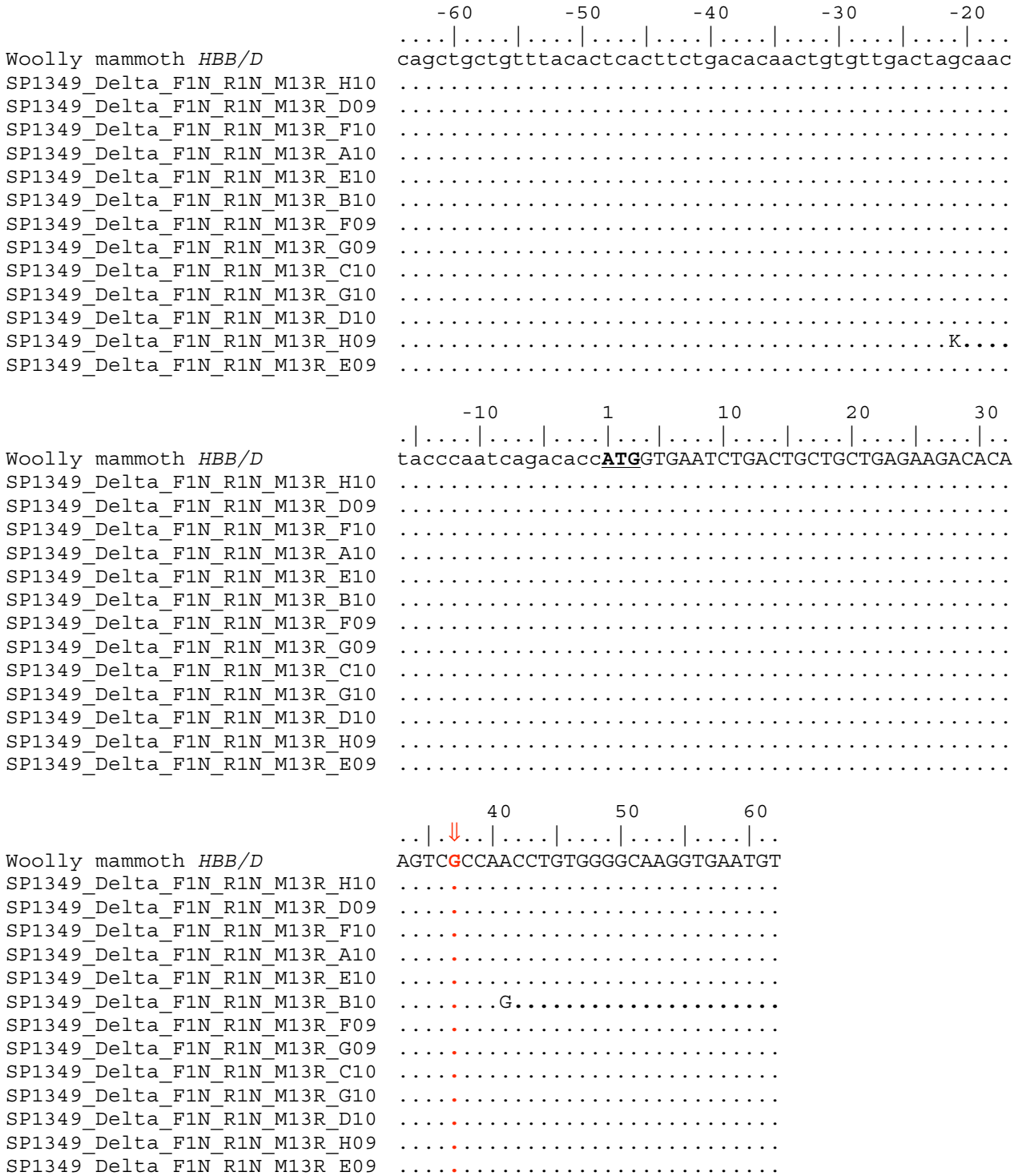
+80 +90 +100 +110 +120

Loxodonta africana |...|...|...|...|...|...|...|...|...|..
 CCAACTCTCAAGGGCATCTCTTCTGCCT**AATAA**AGTACTTTTCAGCTCa

Elephas maximus

CCAACTCTCAAGGCATCTCTTCTGCCTAATAAAAGTACTTTCAGCTCa
'AATAAA'

Supplementary Figure 4. Clones of the woolly mammoth (SP1349) *HBB/D* region including the $\beta/\delta 12Aa$ SNP at nucleotide position 37 (indicated in red), amplified in Adelaide using the SP1349_Delta_F1N/SP1349_Delta_R1N primers (see Supplementary Table 2). Dots indicate identical bases to those found in the direct sequence reads (top line; taken from Supplementary Fig. 3). The small amounts of sequence variation detected in clones is consistent with ancient DNA template damage⁷, but is at sufficiently low levels to be indistinguishable from background enzymatic error rates, which indicates that SP1349 has excellent ancient DNA preservation. Importantly, no variation is detected at the key non-synonymous nucleotide substitution at codon 12.



Supplementary Figure 5. Clones of the woolly mammoth (SP1349) *HBB/D* region covering the $\beta/\delta 86\text{Ser}$ and $\beta/\delta 101\text{Gln}$ SNPs at nucleotide positions 259 and 304, respectively (indicated in red), amplified in Adelaide using the primers SP1349_Delta_F4.1N/ SP1349_Delta_R4 (see Supplementary Table 2). Dots indicate identical bases to those found in the direct sequence reads (top line; taken from Supplementary Fig. 3). The very small amounts of sequence variation within the clones are consistent with ancient DNA damage (G A/C T transition substitutions)⁷, and are indistinguishable from background enzymatic error rates. This confirms that the preservation of DNA in SP1349 is of very high quality. Importantly, no variation is detected around the non-synonymous nucleotide substitutions at codons 86 and 101.

```

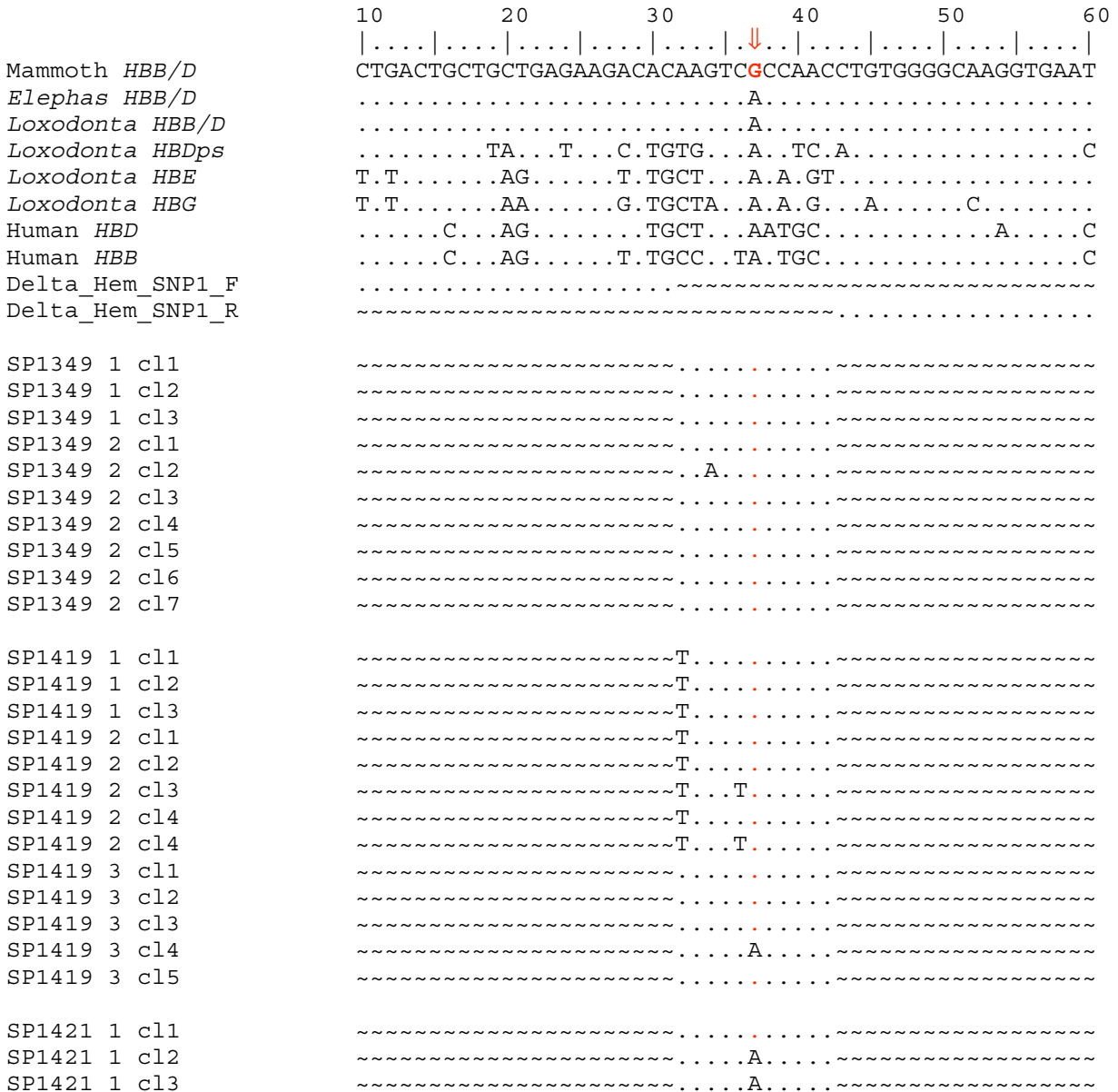
181          190          200          210          220          230
...|...|...|...|...|...|...|...|...|...|...|
Woolly mammoth HBB/D      GTGCTGGCCCATGGCGAGAAAAGTGTGACCTCCTTTGGTGAGGGCCTGAA
SP1349_F4.1N_R4_M13R_A01  .....A.....
SP1349_F4.1N_R4_M13R_E02  .....
SP1349_F4.1N_R4_M13R_G02  .....
SP1349_F4.1N_R4_M13R_C01  .....
SP1349_F4.1N_R4_M13R_E01  .....
SP1349_F4.1N_R4_M13R_G01  .....
SP1349_F4.1N_R4_M13R_D01  .....
SP1349_F4.1N_R4_M13R_F02  .....
SP1349_F4.1N_R4_M13R_H01  .....
SP1349_F4.1N_R4_M13R_F01  .....

          240          250          260          270          280
...|...|...|...|...|...|...|...|...|...|...|
Woolly mammoth HBB/D      GCACCTGGACAACCTCAAGGGCACCTTTTCCGATCTGAGCGAGCTGCACT
SP1349_F4.1N_R4_M13R_A01  .....
SP1349_F4.1N_R4_M13R_E02  .....
SP1349_F4.1N_R4_M13R_G02  .....
SP1349_F4.1N_R4_M13R_C01  .....
SP1349_F4.1N_R4_M13R_E01  .....
SP1349_F4.1N_R4_M13R_G01  .....T.....
SP1349_F4.1N_R4_M13R_D01  .....
SP1349_F4.1N_R4_M13R_F02  .....
SP1349_F4.1N_R4_M13R_H01  .....
SP1349_F4.1N_R4_M13R_F01  .....

          290          300          310
...|...|...|...|...|...|...|...|...|...|...|
Woolly mammoth HBB/D      GTGACAAGCTGCACGTGGATCCTCAGAAATTCAGGgtgagtctaggaga
SP1349_F4.1N_R4_M13R_A01  .....
SP1349_F4.1N_R4_M13R_E02  .....
SP1349_F4.1N_R4_M13R_G02  .....
SP1349_F4.1N_R4_M13R_C01  .....
SP1349_F4.1N_R4_M13R_E01  .....
SP1349_F4.1N_R4_M13R_G01  .....
SP1349_F4.1N_R4_M13R_D01  .....
SP1349_F4.1N_R4_M13R_F02  .....
SP1349_F4.1N_R4_M13R_H01  .....
SP1349_F4.1N_R4_M13R_F01  .....

```

Supplementary Figure 6. SNP targeting of the woolly mammoth specific A G substitution at codon 12 (Leipzig). Two to three independent PCR reactions were conducted on DNA extracted from three mammoth specimens. Note: SP1349 is the individual from which the complete *HBA-T2* and *HBB/D* coding sequences were determined (Supplementary Figs. 2–5). Clone sequences without primers (Delta_Hem_SNP1_F and Delta_Hem_SNP1_R) are given; dots indicate identical bases to those found in the woolly mammoth *HBB/D* gene (see Supplementary Fig. 3). The mammoth specific A G substitution at position 37 is indicated in red and was consistently recovered in the clones from PCR amplicons of specimens SP1349 and SP1419. Notably, this substitution was also detected in a 454 sequence read from specimen M4 (GenBank SRR006688.110153), which was the primary source of the complete mammoth nuclear genome sequence⁸. The expected G was also found in all three PCR reactions conducted on specimen SP1421, although exceptions were found in the first two PCR experiments. Such sequence variation is commonly observed in clones of PCR products from ancient specimens, and generally represents transition mutations characteristic of cytosine deamination on one or other of the source template strands⁷. This pattern is common in ancient DNA when small numbers of templates initiate PCR reactions, and the resulting clones exhibit high frequencies of G A/C T transition substitutions at a few positions⁷. The amount of sequence variation in the clones from the three specimens indicate that the DNA preservation in SP1349 is considerably better than the other two. Importantly, all “blank” (no DNA) negative controls revealed no sign of contamination.



SP1421	1	c14	~~~~~.A.~~~~~
SP1421	2	c11	~~~~~.A.~~~~~
SP1421	2	c12	~~~~~.~~~~~
SP1421	2	c13	~~~~~. A. A.~~~~~
SP1421	2	c14	~~~~~.~~~~~
SP1421	2	c15	~~~~~.~~~~~
SP1421	2	c16	~~~~~. A. A.~~~~~
SP1421	2	c17	~~~~~. A. A.~~~~~
SP1421	3	c11	~~~~~.~~~~~
SP1421	3	c12	~~~~~.~~~~~
SP1421	3	c13	~~~~~.~~~~~
SP1421	3	c14	~~~~~.~~~~~

Supplementary Figure 7. SNP targeting of the woolly mammoth specific G T substitution at codon 86 (Leipzig). Unfortunately, no sequence reads from the mammoth genome project⁸ covered this region. Anywhere from two to five independent PCR reactions were conducted on DNA extracted from four mammoth specimens. Note: SP1349 is the individual from which the complete *HBA-T2* and *HBB/D* coding sequences were determined (Supplementary Figs. 2–5). Clone sequences without primers (Delta_Hem_SNP2_F and Delta_Hem_SNP2_R) are given; dots indicate identical bases to those found in the woolly mammoth *HBB/D* gene (see Supplementary Fig. 3). The mammoth specific G T substitution at position 259 is indicated in red, and was consistently obtained from all clones of SP1349. However, numerous clones from the two less well-preserved specimens possessed “G” at this site, as observed in the *Loxodonta* and *Elephas HBB/D* gene, and the *Loxodonta* gamma (*HBG*), epsilon (*HBE*) and beta pseudogene (*HBBps*) loci. Since all “blank” (no DNA) negative controls revealed no sign of contamination, it appeared likely that one of the mammoth β -cluster paralogs had been co-amplified in the PCR reaction, due to the lack of suitable templates in the early rounds of the PCR. This result is also possibly exaggerated because it is very difficult to design gene-specific primers within the exon regions used here (as opposed to the larger amplifications performed in Adelaide; Supplementary Figs. 1–5 above). To investigate this issue further, and determine which of the β -cluster loci had amplified, a second reverse primer (Delta_Hem_SNP2_R_new; Table S3) was designed to amplify several distinguishing SNPs immediately outside the initially targeted region, and used on two problematic mammoth samples (SP1420, SP1421). The sequences of these clones (shown below the clones for the same specimen) show that the most likely explanation is that in addition to the target *HBB/D* locus, the mammoth *HBG* gene had co-amplified in some of the PCRs of both SP1419 and SP1421. It is important to note that since changes in the flanking region sequences mean that the primers would strongly preferentially bind to any authentic *HBB/D* loci that were present, if there had been any modern elephant contamination present it would have been highly likely to result in elephant *HBB/D* sequences (*i.e.*, not *HBG* or *HBE* paralogs). However, we also cannot entirely discount the possibility that these two individuals are heterozygotic at this site.

	230	240	250	260	270	280
Mammoth <i>HBB/D</i>	AAGCACCTGGACAACCTCAAGGGCACCTTTT	↓	CCGATCTGAGCGAGCTGCACTGTG		
<i>Elephas HBB/D</i>	G
<i>Loxodonta HBB/D</i>	G
<i>Loxodonta HBDps</i>	. . T A C . G T
<i>Loxodonta HBBps</i> T . . T . . G G . . C . G . . A . . TA
<i>Loxodonta HBE</i>	. . . A . A A . . G G . TA . G
<i>Loxodonta HBG</i>	. . . A G . TA . G
Human <i>HBD</i>	GCT	T TC . G T
Human <i>HBB</i>	GCT G . ACA T
Delta_Hem_SNP2_F	~
Delta_Hem_SNP2_R	~
D_Hem_SNP2_R_new	~
SP1349 1 c11	~
SP1349 1 c12	~
SP1349 1 c13	~
SP1349 1 c14	~
SP1349 1 c15	~
SP1349 2 c11	~
SP1349 2 c12	~
SP1349 2 c13	~
SP1349 2 c14	~
SP1349 2 c15	~
SP1349 2 c16	~
SP1349 3 c11	~
SP1349 3 c12	~
SP1419 1 c16	~
SP1419 2 c11	~

```
SP1419 2 c16 ~~~~~ . . . . . ~~~~~
SP1419 3 c13 ~~~~~ . . . . . ~~~~~
SP1419 3 c16 ~~~~~ . . . . . ~~~~~
SP1419 1 c11 ~~~~~ . . . . . G. ~~~~~
SP1419 1 c12 ~~~~~ . . . . . G. ~~~~~
SP1419 1 c13 ~~~~~ . . . . . G. ~~~~~
SP1419 1 c14 ~~~~~ . . . . . GT ~~~~~
SP1419 1 c15 ~~~~~ . . . . . G. ~~~~~
SP1419 1 c17 ~~~~~ . . . . . G. ~~~~~
SP1419 2 c12 ~~~~~ . . . . . G. ~~~~~
SP1419 2 c13 ~~~~~ . . . . . G. ~~~~~
SP1419 2 c14 ~~~~~ . . . . . G. ~~~~~
SP1419 2 c15 ~~~~~ . . . . . G. ~~~~~
SP1419 2 c17 ~~~~~ . . . . . T . . . . . G. ~~~~~
SP1419 3 c11 ~~~~~ . . . . . G. ~~~~~
SP1419 3 c12 ~~~~~ . . . . . G. ~~~~~
SP1419 3 c14 ~~~~~ . . . . . G. ~~~~~
SP1419 3 c15 ~~~~~ . . . . . G. ~~~~~

SP1420 new c11 ~~~~~ . . . . . G.TA.G. ~~~~~
SP1420 new c12 ~~~~~ . . . . . G.TA.G. ~~~~~
SP1420 new c13 ~~~~~ . . . . . G.TA.G. ~~~~~
SP1420 new c14 ~~~~~ . . . . . G.TA.G. ~~~~~

SP1421 1 c12 ~~~~~ . . . . . . . ~~~~~
SP1421 1 c19 ~~~~~ . . . . . . . ~~~~~
SP1421 1 c111 ~~~~~ . . . . . . . ~~~~~
SP1421 1 c112 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c11 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c12 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c13 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c14 ~~~~~ . . . . . . T ~~~~~
SP1421 4 c15 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c16 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c17 ~~~~~ . . . . . . . ~~~~~
SP1421 4 c18 ~~~~~ . . . . . . . ~~~~~
SP1421 1 c11 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c13 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c14 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c15 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c16 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c17 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c18 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c110 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c113 ~~~~~ . . . . . G. ~~~~~
SP1421 1 c114 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c11 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c12 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c13 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c14 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c15 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c16 ~~~~~ . . . . . G. ~~~~~
SP1421 2 c17 ~~~~~ . . . . . G. ~~~~~
SP1421 3 c11 ~~~~~ . . . . . G. ~~~~~
SP1421 3 c12 ~~~~~ . . . . . G. ~~~~~
SP1421 3 c13 ~~~~~ . . . . . G. ~~~~~
SP1421 3 c14 ~~~~~ . . . . . A . . . . . G. ~~~~~
SP1421 3 c15 ~~~~~ . . . . . G. ~~~~~
SP1421 3 c16 ~~~~~ . . . . . G. ~~~~~
SP1421 3 c17 ~~~~~ . . . . . G. ~~~~~
SP1421 5 c11 ~~~~~ . . . . . G. ~~~~~
SP1421 5 c12 ~~~~~ . . . . . G. ~~~~~
SP1421 5 c13 ~~~~~ . . . . . G. ~~~~~
```

```
SP1421 5 c14 ~~~~~.G.~~~~~
SP1421 5 c15 ~~~~~.G.~~~~~
SP1421 5 c16 ~~~~~.G.~~~~~
SP1421 5 c17 ~~~~~.G.~~~~~
SP1421 5 c18 ~~~~~.G.~~~~~
SP1421 5 c19 ~~~~~.G.~~~~~
SP1421 new 1 c11 ~~~~~.G.TA.G.~~~~~
SP1421 new 1 c12 ~~~~~.G.TA.G.~~~~~
SP1421 new 1 c13 ~~~~~.A.~~~~~.G.TA.G.~~~~~
SP1421 new 1 c14 ~~~~~.G.TA.G.~~~~~
SP1421 new 2 c11 ~~~~~.G.TA.G.~~~~~
SP1421 new 2 c12 ~~~~~.G.TA.G.~~~~~
```

Supplementary Figure 8. SNP targeting of the woolly mammoth specific G C substitution at codon 101 (Leipzig). Unfortunately, no sequence reads from the mammoth genome project⁸ covered this region. Three independent PCR reactions were conducted on DNA extracted from each of three mammoth specimens. Note: SP1349 is the individual from which the complete *HBA-T2* and *HBB/D* coding sequences were determined (Supplementary Figs. 2–5). Clone sequences without primers (Delta_Hem_SNP3_F and Delta_Hem_SNP3_R) are given; dots indicate identical bases to those found in the woolly mammoth *HBB/D* gene (see Supplementary Fig. 3). The mammoth specific G C substitution at position 304 is indicated in red and was consistently obtained from all clones of SP1349, and was present in clones of both the less well-preserved mammoth specimens (SP1419 and SP1421). However, numerous clones of the latter two also possessed sequences consistent with the *Loxodonta HBBps*, *HBE*, and *HBG* loci, potentially indicating that other genes from the mammoth β -globin cluster had been co-amplified. However, further investigation revealed that dog and human contaminants detected in four separate ‘blank’ negative controls (Blk) were a perfect match for the sequence variants, strongly suggesting that they were in fact a result of contamination. It is important to note that since changes in the flanking region sequences around the genes in the β -globin cluster mean that the primers would strongly preferentially bind to any authentic *HBB/D* loci that were present, so that if any modern elephant contamination were present it would have been highly likely to result in elephant *HBB/D* sequences (*i.e.*, not *HBBps* or *HBE* or *HBG* paralogs) in the SP1349, SP1419 and SP1421 clones; this was not found.

	290	300	310	
	↓	IVS2→
Mammoth <i>HBB/D</i>	CAAGCTGCACGTGGATCCT	C	CAGAATTTTCAGG	gtgagtctaggaga-
<i>Elephas HBB/D</i>	Gc
<i>Loxodonta HBB/D</i>	Gc
<i>Loxodonta HBDps</i>TG	ACt
<i>Loxodonta HBBps</i>A	TGC
<i>Loxodonta HBE</i>	GCC
<i>Loxodonta HBG</i>	GCc
Human HBD	GCc
Human HBB	GCt
Dog HBDCG	CA
Dog HBBCG	CA
Delta_Hem_SNP3_F	~
Delta_Hem_SNP3_R	~c
SP1349 1 c11
SP1349 1 c12
SP1349 1 c13
SP1349 1 c14
SP1349 1 c15
SP1349 2 c11
SP1349 2 c12
SP1349 2 c13
SP1349 2 c14
SP1349 2 c15
SP1349 2 c16
SP1349 3 c11
SP1349 3 c12
SP1349 3 c3
SP1349 3 c4
SP1349 3 c5
SP1419 1 c11
SP1419 1 c13
SP1419 1 c14
SP1419 1 c15
SP1419 1 c18
SP1419 2 c12
SP1419 2 c17

```

SP1419 3 c11      ~~~~~C~~~~~
SP1419 3 c12      ~~~~~C~~~~~
SP1419 3 c14      ~~~~~C~~~~~
SP1419 1 c12      ~~~~~G...C~~~~~
SP1419 1 c16      ~~~~~G...C~~~~~
SP1419 1 c17      ~~~~~G...C~~~~~
SP1419 1 c19      ~~~~~G...C~~~~~
SP1419 1 c110     ~~~~~G...C~~~~~
SP1419 1 c111     ~~~~~G...C~~~~~
SP1419 1 c112     ~~~~~G...C~~~~~
SP1419 2 c11      ~~~~~CG...C~~~~~
SP1419 2 c13      ~~~~~G...C~~~~~
SP1419 2 c14      ~~~~~G...C~~~~~
SP1419 2 c15      ~~~~~G...C~~~~~
SP1419 2 c16      ~~~~~G...C~~~~~
SP1419 2 c18      ~~~~~G...C~~~~~
SP1419 2 c19      ~~~~~G...C~~~~~
SP1419 2 c110     ~~~~~G...C~~~~~
SP1419 2 c111     ~~~~~G...C~~~~~
SP1419 2 c112     ~~~~~G...C~~~~~
SP1419 2 c113     ~~~~~G...C~~~~~
SP1419 2 c114     ~~~~~G...C~~~~~
SP1419 2 c115     ~~~~~G...C~~~~~
SP1419 3 c13      ~~~~~G...C~~~~~
SP1419 3 c15      ~~~~~G...C~~~~~
SP1419 3 c16      ~~~~~G...C~~~~~
SP1419 3 c17      ~~~~~G...C~~~~~

SP1421 1 c11      ~~~~~C~~~~~
SP1421 1 c13      ~~~~~C~~~~~
SP1421 1 c14      ~~~~~C~~~~~
SP1421 3 c11      ~~~~~C~~~~~
SP1421 3 c12      ~~~~~G...C~~~~~
SP1421 3 c13      ~~~~~C~~~~~
SP1421 3 c14      ~~~~~C~~~~~
SP1421 3 c15      ~~~~~C~~~~~
SP1421 3 c16      ~~~~~C~~~~~
SP1421 1 c12      ~~~~~G...C~~~~~
SP1421 2 c11      ~~~~~G...C~~~~~
SP1421 2 c12      ~~~~~G...C~~~~~
SP1421 2 c13      ~~~~~G...C~~~~~
SP1421 2 c14      ~~~~~G...C~~~~~
SP1421 2 c15      ~~~~~G...C~~~~~
SP1421 2 c16      ~~~~~G...C~~~~~
SP1421 2 c17      ~~~~~G...C~~~~~

Blk 1 c11      ~~~~~CG...C~~~~~
Blk 1 c12      ~~~~~CG...C~~~~~
Blk 1 c13      ~~~~~CG...C~~~~~
Blk 1 c14      ~~~~~CG...C~~~~~
Blk 2 c11      ~~~~~G...C~~~~~
Blk 2 c12      ~~~~~G...C~~~~~
Blk 2 c13      ~~~~~G...C~~~~~
Blk 2 c14      ~~~~~G...C~~~~~
Blk 2 c15      ~~~~~G...C~~~~~
Blk 3 c11      ~~~~~G...C~~~~~
Blk 3 c12      ~~~~~G...C~~~~~
Blk 3 c13      ~~~~~G...C~~~~~
Blk 3 c14      ~~~~~G...C~~~~~
Blk 3 c15      ~~~~~G...C~~~~~
Blk 3 c16      ~~~~~G...C~~~~~
Blk 3 c17      ~~~~~G...C~~~~~
Blk 4 c11      ~~~~~CG...C~~~~~
Blk 4 c12      ~~~~~CG...C~~~~~

```

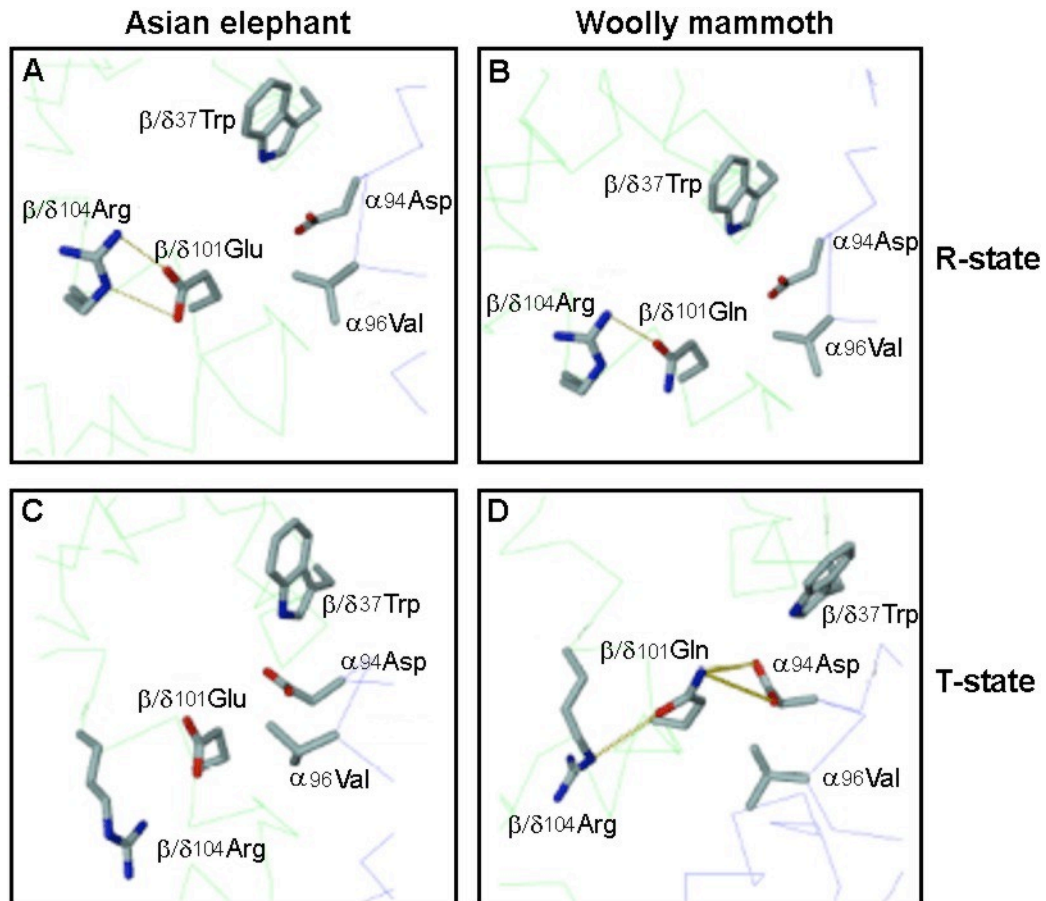
Blk 4 c13	~~~~~CG...C~~~~~
Blk 4 c14	~~~~~CG...C~~~~~
Blk 4 c15	~~~~~CG...C~~~~~
Blk 4 c16	~~~~~CG...C~~~~~

Homo sapiens .H..PE..KA.TA.....D.V....G.....Q...S.....P...MG.P..K...K...GA.SD..A.....AT.....E.....C...H.....P....Q.....
Macaca mulatta .H..PE..SN.TT.....D.V....G...L...Q...S.....SP...MG.P..K...K...GA.SD..N.....AQ.....E..K.....C...H.....Q...Q.....
Ateles geoffroyi .H..GE..AA.TA.....D.V....G.....Q...S.....P...MS.P..K...K...GA.SD..A.....AQ.....E.....C...H.....QL...Q.....
Eulemur fulvus TL.S.E.NAH.TS....D.EKV....G.....Q...S.....SPSA.MG.P..K...K...SA.S...H.....VAQ.....E.....V...E...NA.S.AV...Q.....
Lemur variegates TF..PE.NGH.TS....E.KV....G.....Q...S.....SP..IMG.P..K...K...SA.S...H.....AQ.....VA...E..K.....H...ND.S.QT...FQ...T.....
Tarsius bancanus .H...D..AA.TA....D.EDV....G.....Q...DS.....PA..MG.A..K...K...NA.S..MA.....AK.....E.....C...H.....Q...Q.....T.....
Tupaia glis .H.SGE..AA.TG....DLEKV..QSLGS..I....Q...DS.....SPS..MS.P..K...K...SD..N.....AK.....E.....R...CN..PE...Q...FQ.....

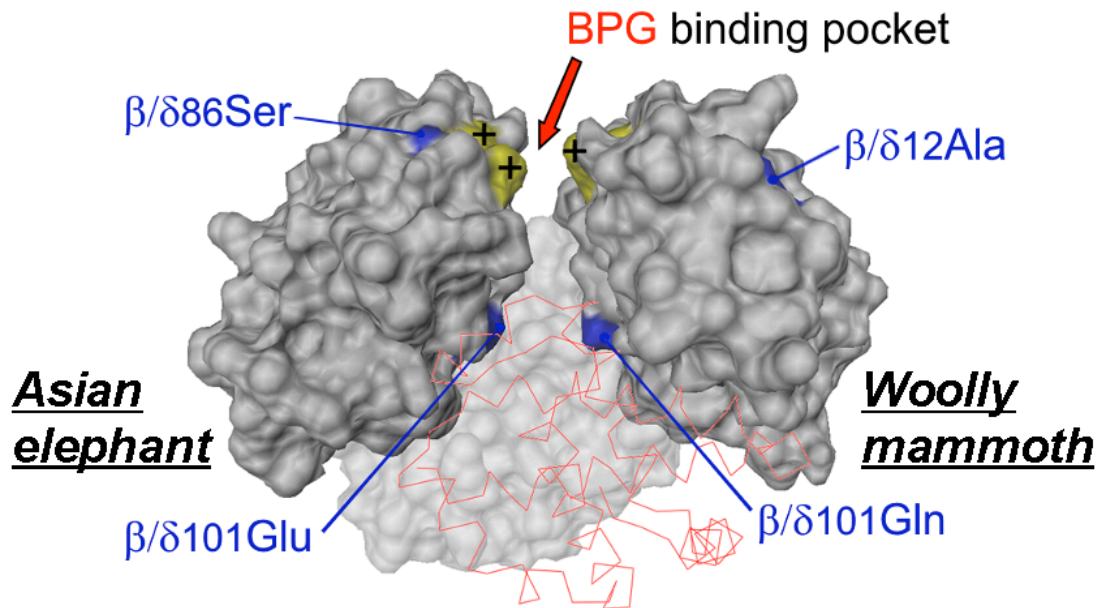
Marsupialia
Didelphis marsupialis .H..SE..NCITTI.S..Q.DQT....G.M.....T...GS.....SPG..MS.S..Q...A.....AV.....YAK.....E..KM...II..C..E...D...EC...WQ.L...H.....
Macropus eugenii .H...E..NAITS....AIEQT....G...I....S...D.....N.K..MS.P....A...VA..DAI.N.....AK.....D.....N.K..E..K...II..C..E...I.A.V.WQ.L.....
Macropus giganteus .H...E..NAITS....VAIEQT....G...I....S...D.....N.K..MA.P....A...VA..DAI.N.....AK.....E..K...II..C..E...I.T.V.WQ.L.....

Monotremata
Tachyglossus aculeatus .H.SGS...A.T...H...N.....G.....Q...S.....S...MG...K...A.....DA..N.....AK.....E..NR....V....S....EA...WQ.L.S..SH.....
Ornithorhynchus anatinus .H.SGG..SA.T.....IN.....G.....Q...A....S.G..MG.P..K...A.....DA..N..D...AK.....E..NR....IV....S.D.S.E...WQ.L.S..H..G...

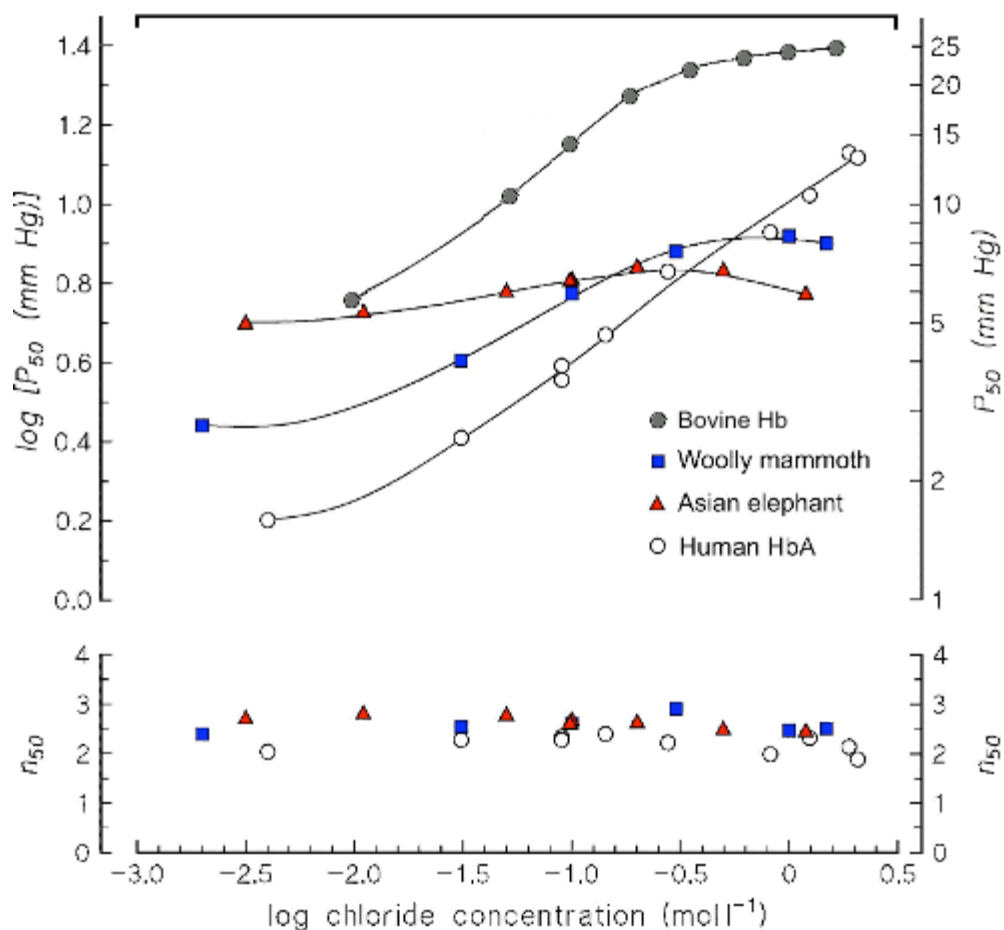
Supplementary Figure 10. Molecular model illustrating altered electrostatic interactions in mammoth hemoglobin (Hb) arising from the β/δ -globin chain E101Q substitution. The carboxyl group of β/δ 101Glu in R state Asian elephant Hb (**A**) interacts closely with the guanidino group of β/δ 104Arg of the same chain. This interaction is weakened in mammoth oxyHb causing the R state to lose one hydrogen bond (**B**). However, in comparison to Asian elephant deoxyHb (**C**), the uncharged β/δ 101Gln residue of mammoth deoxyHb interacts electrostatically with both the N_{ϵ} -nitrogen of β/δ 104Arg and the carboxyl group of α 94Asp (**D**).



Supplementary Figure 11. Side view surface model of a chimeric Asian elephant (left) and woolly mammoth (right) deoxyhemoglobin molecule. The locations of the three mammoth-specific amino-acid substitutions are highlighted in blue, while regions highlighted in yellow denote residues ($\beta/\delta 1$ Val, $\beta/\delta 82$ Lys, $\beta/\delta 143$ His) implicated in 2,3-bisphosphoglycerate (BPG) binding. The mammoth $\beta/\delta 101$ Gln residue is spatially distant from this site and hence does not alter the phosphate sensitivity of the Hb moiety. The mammoth α -globin chain is represented by a backbone diagram.



Supplementary Figure 12. The effect of the chloride ion concentration on the oxygen affinity (half-saturation pressure; P_{50}) and cooperativity coefficients (n_{50}) of woolly mammoth, Asian elephant, bovine and adult human hemoglobin at 25°C and in the absence of 2,3-bisphosphoglycerate. Data from human and bovine Hbs taken from refs 12 and 13, respectively. Mammoth, elephant and human Hb were measured at pH 7.4 while that of bovine Hb was measured at pH 7.2.



Supplementary Table 1. Primary/external primers used to amplify woolly mammoth *HBA-T2* and *HBB/D* globin genes from woolly mammoth specimen SP1349 (Adelaide).

Frag ID	Primer name	5' to 3' sequence	Frag size including primers	Bp of sequence data
HBA-T2 F1	SP1349_Alpha2_F1.1	CGCTTCTGATCTCCACACAGACTC	155	112
	SP1349_Alpha2_R1.3	GTAGGGGACAGGGGGATCC		
HBA-T2 F2	SP1349_Alpha2_F2N	TCCCTGTTCCCCTACCCTAC	100	62
	SP1349_Alpha2_R2.2N	GTGAGGAAAGTAGGTCTT		
HBA-T2 F3	SP1349_Alpha2_F2.3	CACGCAGGATGTTCTTCT	140	104
	SP1349_Alpha2_R2.3N	GTCATCCAGGTGGCCAAC		
HBA-T2 F4	SP1349_Alpha2_F2.4	GAAGGTGGGGGAAGCACTGA	140	102
	SP1349_Alpha2_R2.4N	CCAGGTCTTCCCCTCTCC		
HBA-T2 F5	SP1349_Alpha2_F3N	AGGTGAGCGTTCCTAACAG	117	77
	SP1349_Alpha2_R3.2	CATGGACCTCAGGGGTGAAC		
HBA-T2 F6	SP1349_Alpha2_F3.1N	CTCTCTCCACAGCTCCTG	153	115
	SP1349_Alpha2_R3N	CTGGGGTCCAGCTTAACGAT		
HBA-T2 F7	SP1349_Alpha2_F3.1N	CTCTCTCCACAGCTCCTG	189	---
	SP1349_Alpha2_R3	GGAGGAAGGCCAGTGGCA		
HBB/D F1	SP1349_Delta_F1	CAGGACCAGGGTTGGGCA	209	---
	SP1349_Delta_R1	GCAACCTAGATACAAACCTGCT		
HBB/D F2	SP1349_Delta_F2	GCTGAGAAGACACAAGTCACCAACC	184	---
	SP1349_Delta_R2	CAGGGTGCAGAGGAAGTCAGTGC		
HBB/D F3	SP1349_Delta_F3	TGGGCATGTGGAGACAGAACA	214	---
	SP1349_Delta_R3	AGCCCTCACCAAAGGAGGTC		
HBB/D F4	SP1349_Delta_F4.1	TGACGCTGTCCTGCACAACG	202	---
	SP1349_Delta_R4	CTACAAAGTGAAGAAAGAAAAATAGAATG		
HBB/D F5	SP1349_Delta_F5	TGTATGTATCTACCTCTTCCCCTAG	239	---
	SP1349_Delta_R5	GAGAGTTGGCATTATTTCCCAA		
HBB/D promoter	SP1349_Del_Upstr_F	TGGCTGTCATCATTGAGCC	145	105
	SP1349_Del_Upstr_R	GTTGTGTCAGAAGTGAGTGT		

Supplementary Table 2. Secondary/internal primers used to amplify the woolly mammoth *HBA-T2* and *HBB/D* globin genes from woolly mammoth specimen SP1349 (Adelaide).

Frag ID	Primer name	5' to 3' sequence	Frag size including primers	Bp of sequence data
HBA-T2 F3	SP1349_Alpha2_F2.3N	GTTCTTCTCCTTTCCCACCACCAA	130	88
	SP1349_Alpha2_R2.3N	GTCATCCAGGTGGCCAAC		
HBA-T2 F4	SP1349_Alpha2_F2.4N	GGGAAGCACTGACCCAA	132	97
	SP1349_Alpha2_R2.4N	CCAGGTCTTCCCCTCTCC		
HBA-T2 F5	SP1349_Alpha2_F3N	AGGTGAGCGTTCCTAACAG	112	72
	SP1349_Alpha2_R3.2N	ACCTCAGGGGTGAACTCCGT		
HBA-T2 F6	SP1349_Alpha2_F3.2	GTGACTCTAAGCAGCCACC	120	81
	SP1349_Alpha2_R3N	CTGGGGTCCAGCTTAACGAT		
HBA-T2 F7	SP1349_Alpha2_F3.2	GTGACTCTAAGCAGCCACC	146	107
	SP1349_Alpha2_R3	GGAGGAAGGCCAGTGGCA		
HBB/D F1	SP1349_Delta_F1N	GGGCATATAAGGAAGAGTAGTGC	169	126
	SP1349_Delta_R1N	CCTCACCACCAAGCTCTTTC		
HBB/D F2	SP1349_Delta_F2N	CACAAGTCACCAACCTGTGG	153	113
	SP1349_Delta_R2N	GCCTGTCAGAACTGGGAGA		
HBB/D F2I	SP1349_Delta_F2.2	GACTTAAGGAGGGTTGAGTG	87	44
	SP1349_Delta_R2	CAGGGTGCAGAGGAAGTCAGTGC		
HBB/D F3	SP1349_Delta_F3N	CAGAACAGTCTCCCAGTTTCTG	191	177
	SP1349_Delta_R3N	AAAGGAGGTCAACACTTTCTCG		
HBB/D F4	SP1349_Delta_F4.1N	GCTGTCCTGCACAACGCTAAA	198	149
	SP1349_Delta_R4	CTACAAAGTGAAAAGAAAAAATAGAATG		
HBB/D F5	SP1349_Delta_F5	TGTATGTATCTACCTCTTCCCCATAG	213	166
	SP1349_Delta_R5N	CAGAGATAGCATCTCGGGAAA		

Supplementary Table 3. Primers used to replicate mammoth specific non-synonymous substitutions of the *HBB/D* globin gene (Leipzig).

Frag ID	Primer name	5' to 3' sequence	Frag size including primers	Bp of sequence data
β/δ 12 SNP	Delta_Hem_SNP1_F	CTGACTGCTGCTGAGAAGACAC	51	11
	Delta_Hem_SNP1_R	ATTCACCTTGCCCCACAG		
β/δ 86 SNP	Delta_Hem_SNP2_F	AGCACCTGGACAACCTCAAG	48	11
	Delta_Hem_SNP2_R	GCAGCTCGCTCAGATCG	54	16
	Delta_Hem_SNP2_R_new	CACAGTGCAGCTCGCTCA		
β/δ 101 SNP	Delta_Hem_SNP3_F	CAAGCTGCACGTGGATCC	46	7
	Delta_Hem_SNP3_R	GTCTCCTAGACTCACCCCTGAA		

Supplementary Table 4. Primer pairs used to convert the Asian elephant pHE27E plasmid into the mammoth pHE27M plasmid via site-directed mutagenesis. The mutated codons are underlined.

Amino-acid change	5' to 3' sequence
K5N of α -chain	GGAGGAATAACATATGGTCTTAAGTGATA <u>AAC</u> GACAAGACCAACGTCAAG
	CTTGACGTTGGTCTTGTGCGTTATCACTTAAGACCATATGTTATTCTCC
T12A of β/δ -chain	GCTGAGAAGACACAAGTG <u>GCC</u> AACCTGTGGGGCAAGG
	CCTTGCCCCACAGGTTGGCCACTTGTGTCTTCTCAGC
A86S of β/δ -chain	CTGGACAACCTCAAGGGTACCTTTT <u>TCC</u> GATCTGAGCGAGCTG
	CAGCTCGCTCAGATCGGAAAAGGTACCCTTGAGGTTGTCCAG
E101Q of β/δ -chain	GACAAGCTGCACGTGGACCCT <u>CAG</u> AATTCAGGCTCCTG
	CAGGAGCCTGAAATTCTGAGGGTCCACGTGCAGCTTGT

Supplementary Information References

1. Hardies, S. C., Edgell, M. H. & Hutchison III, C. A. Evolution of the mammalian beta-globin gene cluster. *J. Biol. Chem.* **259**, 3748-3756 (1984).
2. Hoffmann, F. G., Opazo, J. C. & Storz, J. F. Rapid rates of lineage-specific gene duplication and deletion in the alpha-globin gene family. *Mol. Biol. Evol.* **25**, 591-602 (2008).
3. Aguilera, G., Bielawski, J. P. & Yang, Z. Proposed standard nomenclature for the α - and β -globin gene families. *Genes Genet. Syst.* **81**, 367-371 (2006).
4. Garrick, M. D. & Garrick, L. M. in *Red Blood Cells of Domestic Mammals* (eds Agar N. S. & Board, P. G.) 165-207 (Elsevier, Amsterdam, 1983).
5. Myers, R. M., Tilly, K. & Maniatis, T. Fine structure genetic analysis of a beta-globin promoter. *Science* **232**, 613-618 (1986).
6. Opazo, J. C., Sloan, A. M., Campbell, K. L. & Storz, J. F. Origin and ascendency of a chimeric fusion gene: the beta/delta-globin gene of paenungulate mammals. *Mol. Biol. Evol.* **26**, 1469-1478 (2009).
7. Brotherton, P. *et al.* Novel high-resolution characterization of ancient DNA reveals C>U-type base modification events as the sole cause of *post mortem* miscoding lesions. *Nucleic Acids Res.* **35**, 5717-5728 (2007).
8. Miller, W. *et al.* Sequencing the nuclear genome of the extinct woolly mammoth. *Nature* **456**, 387-390 (2008).
9. Clementi, M. A., Condò, S. G., Castagnola, M. & Giardina, B. Hemoglobin function under extreme life conditions. *Eur. J. Biochem.* **223**, 309-317 (1994).
10. De Rosa M. C., Castagnola, M., Bertonati, C., Galtier, A. & Giardina, B. From the Arctic to fetal life: physiological importance and structural basis of an 'additional' chloride-binding site in haemoglobin. *Biochem. J.* **380**, 889-896 (2004).
11. Fronticelli, C. *et al.* Allosteric modulation by tertiary structure in mammalian hemoglobins. Introduction of the functional characteristics of bovine hemoglobin into human hemoglobin by five amino acid substitutions. *J. Biol. Chem.* **270**, 30588-30592 (1995).
12. Weber, R.E. Use of ionic and zwitterionic (Tris/BisTris and HEPES) buffers in studies on hemoglobin function. *J. Appl. Physiol.* **72**, 1611-1615 (1992).
13. Perutz, M. F., Fermi, G., Poyart, C., Pagnier, J. & Kister, J. A novel allosteric mechanism in haemoglobin: structure of bovine deoxyhaemoglobin, absence of specific chloride-binding sites and origin of the chloride-linked Bohr effect in bovine and human haemoglobin. *J. Mol. Biol.* **233**, 536-545 (1993).