advances.sciencemag.org/cgi/content/full/3/7/e1602878/DC1

Supplementary Materials for

Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades

Michael J. Gaudry, Martin Jastroch, Jason R. Treberg, Michael Hofreiter, Johanna L. A. Paijmans, James Starrett, Nathan Wales, Anthony V. Signore, Mark S. Springer, Kevin L. Campbell

Published 12 July 2017, *Sci. Adv.* **3**, e1602878 (2017)
DOI: 10.1126/sciadv.1602878

The PDF file includes:

- fig. S1. Schematic maximum-likelihood tree of *ucp* sequences used in this study ($n = 400$).
- fig. S2. Exon alignments depicting deleterious mutations found in *UCP1* sequences of placental mammal taxa.
- fig. S3. Ribbon diagram of residues 13 to 304 of human UCP1 (UniProt accession number P25874) structurally modeled by SWISS-MODEL.
- fig. S4. Amino acid alignment of vertebrate sln.
- table S1. GenBank accession numbers of species used in this study.
- table S2. Likelihood ratio tests for *ucp1* CODEML models.
- table S3. Calculated *ucp1* inactivation dates (Ma) within the placental mammal lineage.
- table S4. Results of BiSSE models with and without constraints on the diversification rate for five trees from the work of Faurby and Svenning (*56*).
- table S5. BiSSE results for neutral character simulations.
- table S6. Deleterious mutations found in *ucp2* and *ucp3* sequences of placental mammal taxa.
- table S7. Specimen data and sources of tissue samples used for PCR amplification and DNA hybridization capture.
- table S8. Fossil constraints used in timetree analysis.

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/7/e1602878/DC1)

- data file S1. Gene partitions, a maximum likelihood phylogram, and mcmctree (text file).

- data file S2. *ucp1* free ratio model (text file).
- data file S3. Fossil body mass, taxon diversity, and UCP1 diversification codings (Excel file).
- data file S4. *ucp2* free ratio model (Text file).
- data file S5. *ucp3* free ratio model (Text file).
- data file S6. *ucp1* coding sequence alignment (Fasta file).
- data file S7. *ucp2* coding sequence alignment (Fasta file).
- data file S8. *ucp3* coding sequence alignment (Fasta file).
- data file S9. Accession numbers for the 41 loci used to construct the 51-kb maximum likelihood species tree (Fasta file).
- data file S10. A 51-kb alignment (41 genes) for 140 vertebrate species (Text file).

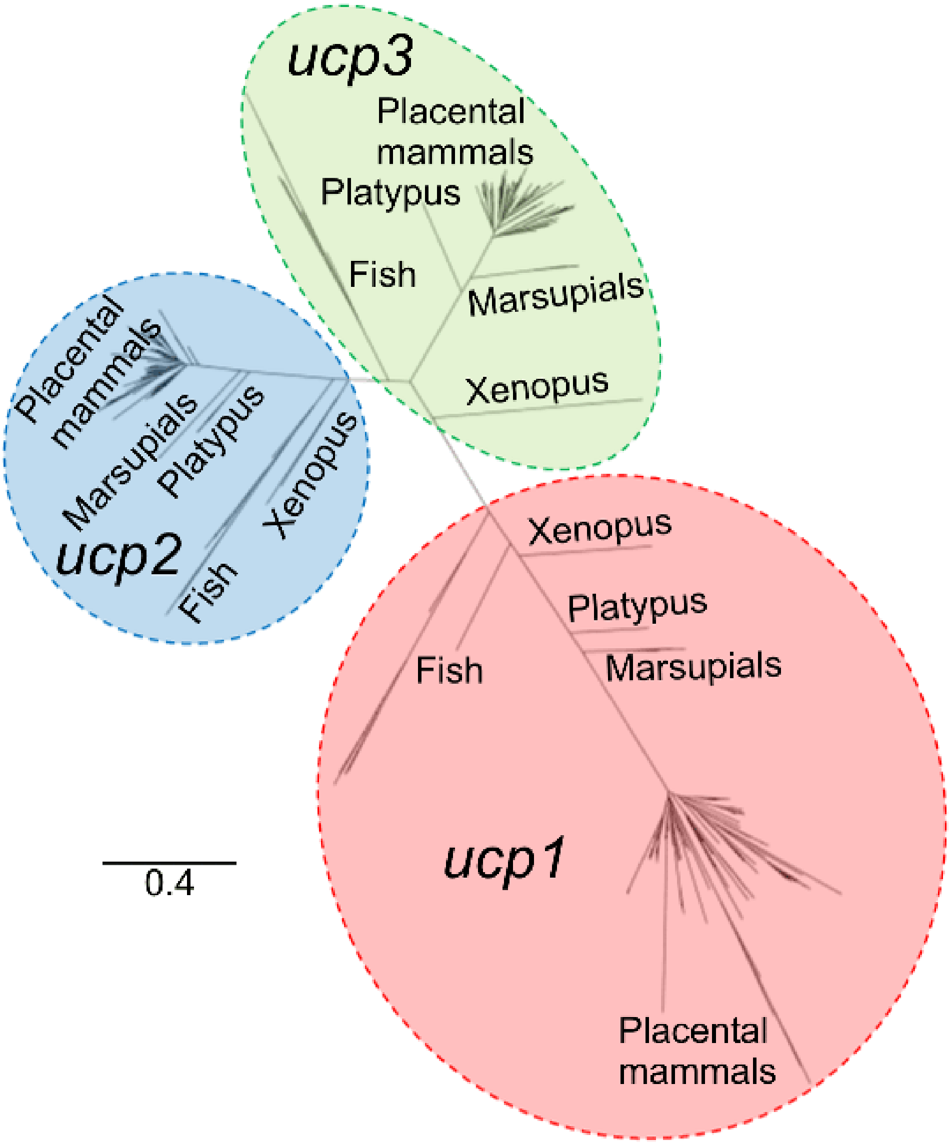

fig. S1. Schematic maximum-likelihood tree of *ucp* sequences used in this study (*n* = 400). Branch lengths denote number of substitutions per site.

Exon 1

```
                                1        10        20        30        40        50
                                |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                    ATGGGGGGCCTGA-CAGCCTCGGACGTACACCCGACCC---TGGGGGTCC
Bradypus variegatus             ??????????????????????????????????????????????????
Choloepus hoffmanni             ??????????????????????????????????????????????????
Mylodon darwinii                ??????????????????????????????????????????????????
Cyclopes didactylus             ??????????????????????????????????????????????????
Dasypus novemcinctus            ATGGGGCGCCAGGGCTCCCGCGGGCTCACCCCCCGC--------------
Procavia capensis               CTG--AGTTAAGA-CAACCTCAGAAATGCCGCCTAC-----------GCA
Elephas maximus                 ACGGTAGGCCAGA-CGACCGCAGACGTGCCCCGGACCATGGTGGGGGTCA
Mammuthus primigenius           ACGGTAGGCCAGA-CGACCGCAGACGTGCCCCGGACCATGGTGGGGGTCA
Loxodonta africana              ACCGTAGGCCAGA-CGACCGCAGACGTGCCCCGGACCATGGTGGGGGTCA
Trichechus manatus              ATGGTGGGCCAGA-CTACCTGGGATGTGCCCCCGACCA---TGGGCGTCA
Dugong dugon                    ATGGTGGGCCAGA-CTACCTCGGATGTGCCCCCGACCA---TGGGCGTCA
Hydrodamalis gigas              ATGGTGGGCCAGA-CTACCTCGGATGTGCCCCCGACCA---TGGGCGTCA
Sus scrofa                      CTGTCAGGA-TGA-CAGTTCCTGAAGTGCCCCCGACCA---TAGCGGTCA
Sus verrucosus                  CTGTCAGGA-TGA-CAGTTCCTGAAGTGCCCTCGACCA---TAGCGGTCA
Sus cebifrons                   CTGTCAGGA-TGA-CAGTTCCTGAAGTGCCCTCGACCA---TAGCGGTCA
Physeter macrocephalus          ATGGTGGGACTCG-CAGCCTCATACGTGCCCCCGACCA---TGGCGGTCA
Delphinapterus leucas           --------------------------------------------------
Lipotes vexillifer              ATGGTGGGACTCG-CAGCCTCAGACGTGCCCCCGACCA---TGGCGGTCA
Balaena mysticetus              ATGGTGGCACTCA-CAGCCTCAGACGTGCCCCCGACCA---TGGCGGTCA
Balaenoptera acutorostrata      ATGCTGGGACTCA-CAGCCTCAGACGCGCCCCCGACCG---TGGCGGTCA
Balaenoptera physalus           ATGCTGGGACTCA-CAGCCTCAGACGCGCCCCCGACCA---TGGCGGTCA
Balaenoptera bonaerensis        ATGCTGGGACTCA-CAGCCTCAGACGCGCCCCCGACCA---TGGCGGTCA
Equus asinus                    ATGGTGGGGCCCA-CAGCCTCGGACCCGCCCCCGACCA---TGGCGGTCA
Equus quagga boehmi             ATGGTGGGGCCCA-CAGCCTCGGACGCGCCCCCGACCA---TGGCGGTCA
Equus przewalskii               ??????????????????????????????????????????????????
Equus caballus                  ATGGTGGGGCCCA-CAGCCTCGGACGTGCCCCCGACCA---TGGCGGTCA
Manis pentadactyla              --------------------------------------------------

                                51       60        70        80        90        100
                                |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                    AGCTCTTCTCAGCTGGA---ATAGCGGCGTGCTTGGCGGACGTGATCACC
Bradypus variegatus             ??????????????????????????????????????????????????
Choloepus hoffmanni             ??????????????????????????????????????????????????
Mylodon darwinii                ??????????????????????????????????????????????????
Cyclopes didactylus             ??????????????????????????????????????????????????
Dasypus novemcinctus            -----------GCGGGAAACGAGGCGGC------GGCAGGTGCACCCCGC
Procavia capensis               ACCTCTTCTCAGCTGGA---GTGGCGGCCTGCTTGGCCGGTGTGATCACC
Elephas maximus                 AGATCTTCTCAGCGGGA---GTGGCGGCCTGCTTGGCAGATGTAATTACC
Mammuthus primigenius           AGATCTTCTCAGCGGGA---GTGGCGGCCTGCTTGGCAGATGTAATTACC
Loxodonta africana              AGATCTTCTCAGCGGGA---GTGGCGGCCTGCTTGGCAGATGTAATTACC
Trichechus manatus              AGATCGTCTCAGCTGGA---GTGTCGGCCTGTTTGGCGGATGTGATCACC
Dugong dugon                    AGATTGTCTCAGCTGGA---GTGTCGGCCTGTTTGGCGGATGTGATCACC
Hydrodamalis gigas              AGATTGTCTCAGCTGGA---GTGTCGGCCTGTTTGGCGGATGTGATCACC
Sus scrofa                      AGATCTTCTCAGCTGGA---GCAGCGGCCTGCGTGGCAGATGTGATCACC
Sus verrucosus                  AGATCTTCTCAGCTGGA---GCGGCGGCCTGCGTGGCAGATGTGATCACC
Sus cebifrons                   AGATCTTCTCAGCTGGA---GCGGCGGCCTGCGTGGCAGATGTGATCACC
Physeter macrocephalus          AGATCTTCTCGGCTGGA---GTGGCGGCCTGCGTGGCGGATGTGATCACC
Delphinapterus leucas           --------------------------------------------------
Lipotes vexillifer              AGATCTTCTCGGCTGGA---GTGGCGGCCAGCGTGGCGGATGTGATCACC
Balaena mysticetus              CGATCTTCTCGGCTGGA---GTGGCGGCCGGCGTGGCGGATGTGATCACC
Balaenoptera acutorostrata      CGATCTTCTCGGCTGGA---GTGGCGGCCGGCGTGGCGGATGTGATCACC
Balaenoptera physalus           CGATCTTCTCGGCTGGA---GTGGCGGCCGGCGTGGCGGATGTGATCACC
Balaenoptera bonaerensis        CGATCTTCTCGGCTGGA---GTGGCGGCCGGCGTGGCGGATGTGATCACC
Equus asinus                    AGATCTTCTCGGCTGGA---GTGGCGGCCTGCGTGGCGGACGTGATCGCC
Equus quagga boehmi             AGATCTTCTCGGCTGGA---GTGGCGGCCTGCGTGGCGGACGTGATCGCC
```

```
Equus przewalskii               ???????????????????????????CCTGCGTGGCGGACGTGATCGCC
Equus caballus                  AGATCTTCTCGGCTGGA---GTGGCGGCCTGCGTGGCGGACGTGATCGCC
Manis pentadactyla              --------------------------------------------------

                                101      110       120       130
                                |...|....|....|....|....|....|....|
Homo sapiens                    TTCCCGC--TGGACACGGCCAAAGTCCGGCTCCAGgt
Bradypus variegatus             ?????????????????????????????????????
Choloepus hoffmanni             ?????????????????????????????????????
Mylodon darwinii                ?????????????????????????????????????
Cyclopes didactylus             ?????????????????????????????????????
Dasypus novemcinctus            CTCCTCCGCTGGG-GCCGCCAGCCTGGGGCTGCAGct
Procavia capensis               TTTCCAC--TGGACACTGCCCAAGTTCGGGTACAGgc
Elephas maximus                 TTCCCGC--TGATCGCGGCCAAAGTTCGGCTGCAGat
Mammuthus primigenius           TTCCCGC--TGGTCGCGGCCAAAGTTCGGCTGCAGat
Loxodonta africana              TTCCCGC--TGGTCGCGGCCAAAGTTCGGCTGCAGat
Trichechus manatus              TTCCCAC--TGGACACGGCCAAGGTTCGGCTACAGgt
Dugong dugon                    TTCCCAC--TGGACACGGCCAAGGTTCGGCTACAGgt
Hydrodamalis gigas              TTCCCAC--TGGACACGGCCAAGGTTCGGCTACAGgt
Sus scrofa                      TTTGCACTTTGGACACCGCCAAAGTCCGGCTACAGgt
Sus verrucosus                  TTTGCACTTTGGACACCGCCAAAGTCCGGCTACAGgt
Sus cebifrons                   TTTGCACTTCGGACACCGCCAAAGTCCGGCTACAGgt
Physeter macrocephalus          TTCCCGC--TGGACACGGCCAAAGTCCGGCTACAGgg
Delphinapterus leucas           -------------------------------------
Lipotes vexillifer              TTCCCGC--TGGACACGGCCAAAGTCCGGCTACAGgg
Balaena mysticetus              TTCCCGC--TGGACACGGCCAAAGTCCGGCTACAGgt
Balaenoptera acutorostrata      TTCCCGC--TGGACACGGCCATAGTCCGGCTACAGgt
Balaenoptera physalus           TTCCCGC–-TGGACACGGCCAAAGTCCGGCTACAGgt
Balaenoptera bonaerensis        TTCCCGC--TGGACACGGCCATAGTCCGGCTACAGgt
Equus asinus                    TTCCCGC--TGGACACGGCCAAAGTCCGGCTGCAGgt
Equus quagga boehmi             TTCCCGC--TGGACACGGCCAAAGTCCGGCTGCAGgt
Equus przewalskii               TTCCCGC--TGGACACGGCCAAAGTCCGGCTGCAGgt
Equus caballus                  TTCCCGC--TGGACACGGCCAAAGTCCGGCTGCAGgt
Manis pentadactyla              -------------------------------------
```

Exon 2

```
                                136  140       150       160       170       180
                                |....|....|....|....|....|....|....|....|....|....|
Homo sapiens                  agGTCCAAGGTGAATGCCCGACGTCCAGTGTTATTAGGTATAAAGGTGTCCTG
Bradypus variegatus           ?????????????????????????????????????????????????????
Choloepus hoffmanni           ?????????????????????????????????????????????????????
Mylodon darwinii              ?????????????????????????????????????????????????????
Cyclopes didactylus           ?????????????????????????????????????????????????????
Dasypus novemcinctus          agATCCAAGGTGAAGGTGAGAAATCCCATGCTATTAAGTACAAAGGTCTCCTG
Procavia capensis             agATCCAAGGTGAATGGCAGAACTCCAGTGCCCTTAGCTATAAAGGCGTCTTG
Elephas maximus               agATCCAAGGTGAATGCCAGACCTCCAGTGCCATTAGGTATAAAGGTGTCTTG
Mammuthus primigenius         agATCCAAGGTGAATGCCAGACCTCCAGTGCCATTAGGTATAAAGGTGTCTTG
Loxodonta africana            agATCCAAGGTGAATGCCAGACCTCCAGTGCCATTAGGTATAAAGGTGTCCTG
Trichechus manatus            agATCCAAGGTGAATGCCAGACCTCCAGTGCCATTAGGTATAAAGTTGTCTTG
Dugong dugon                  agATCCAAGGTGAATGCCAGACCCCCAGTGCCATTAGGTATAAAGGTGTCTTG
Hydrodamalis gigas            agATCCCAGGTGAGTGCCAGACCTCCAGTGCCATTAGGTATAAAGGTGTCTTG
Sus scrofa                    agATCCAAGGCAAATGCCGGTCCTCCGGTACCATTCGGTACAAAGACGTCCTG
Sus verrucosus                agATCCAAGGCAAATGCCGGTCCTCCGGTACCATTCGGTACAAAGACGTCCTG
Sus cebifrons                 agATCCAAGGCAAATGCCGGTCCTCCGGTACCATTCGGTACAAAGACGTCCTG
Physeter macrocephalus        agATCCAATGCGAATGCCAGACCTCCAGTGCTGTTAGGTATAAAAGTGTCCTG
Delphinapterus leucas         -----------------------------------------------------
Lipotes vexillifer            agATCCAATGCGAATGCCAGACCTCCAGTGCTGTTAGGTATAAAAGTGTCCTG
Balaena mysticetus            agATCCAATGCGAATGCCAGATCTCCAGTGCTGTTAGGTATAAAAGTGTCCTG
Balaenoptera acutorostrata    agGTCCAATGCGAATGCCAGACCTCCAGTGCTGTTAGGTATAAAAGTGTCCTG
```

```
Balaenoptera physalus         agATCCAATGCGAATGCCAGACCTCCAGTGCTGTTAGGTATAAAAGTGTCCTG
Balaenoptera bonaerensis      agATCCAATGCGAATGCCAGACCTCCAGTGCTGTTAGGTATAAAAGTGTCCTG
Equus asinus                  agATCCAAGGCGAGCGCCAGACCTCCAGTGCCCTGAGGTATAACGGTATCCTG
Equus quagga boehmi           agATCCAAGGCGAGCGCCAGACCTCCAGTGCCCTGAGGTATAACGGTATCCTG
Equus przewalskii             agATCCAAGGCGAGCGCCAGACCTCCAGTGCCCTGAGGTATAAAGGTATCCTG
Equus caballus                agATCCAAGGCGAGCGCCAGACCTCCAGTGCCCTGAGGTATAAAGGTATCCTG
Manis pentadactyla            -----------------------------------------------------

                              186 190       200       210       220       230
                              |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                  GGAACAATCACCGCTGTGGTAAAAACAGAAGGGCGGATGAAACTCTACAG
Bradypus variegatus           ??????????????????????????????????????????????????
Choloepus hoffmanni           ??????????????????????????????????????????????????
Mylodon darwinii              ??????????????????????????????????????????????????
Cyclopes didactylus           ??????????????????????????????????????????????????
Dasypus novemcinctus          GGAACAGTCGCCACACTGGCAAAAACGGAAGGGCCAATGAA--CCTACCG
Procavia capensis             GAAACAACCACCACCCTGACAAAAACAGAGGGGCCAATGAAACTATAGCT
Elephas maximus               GGAACAATCACTACTCTGGCAAAAAAGGAAGAGCCAATGAAACTCTATAG
Mammuthus primigenius         GGAACAATCACTACTCTGGCAAAAAAGGAAGAGCCAATGAAACTCTATAG
Loxodonta africana            GGAACAATCACTACTCTGGCAAAAAAGGAAGAGCCAATGAAACTCTATAG
Trichechus manatus            GGAACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACGCTATAG
Dugong dugon                  GGAACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACGCTATAG
Hydrodamalis gigas            GGAACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACGCTATAG
Sus scrofa                    GGAACAATCACTACTCTGGCAAAGACAGAAGGGCCAATGAAGCTCTACAG
Sus verrucosus                GGAACAATCACTACTCTGGCAAAGACAGAAGGGCCAATGAAGCTCTACAG
Sus cebifrons                 GGAACAATCACTACTCTGGCAAAGACAGAAGGGCCAATGAAGCTCTACAG
Physeter microcephalus        GGAACAATCACCAC--TGGCAAAAACAAGAGGGCCAATGAAACTCTACAG
Delphinapterus leucas         --------------------------------------------------
Lipotes vexillifer            GGAACAATCACCACTCTGGCAAAAACAAGAGGGCCAATGAAACGCTCCAG
Balaena mysticetus            GGAACAATCACCACTCTGGCAAAAACAAGAGGGCCAATGAAACTCTACAG
Balaenoptera acutorostrata    GGAACAATCACCACTCTGGCAAAAACAAGAGGGCCAATGAAACTCTACAA
Balaenoptera physalus         GGAACAATCACCACTCTGGCAAAAACAAGAGGGCCAATGAAACTCTACAG
Balaenoptera bonaerensis      GGAACAATCACCACTCTGGCAAAAACAAGAGGGCCAATGAAACTCTACAA
Equus asinus                  GGGACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
Equus quagga boehmi           GGGACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
Equus przewalskii             GGGACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
Equus caballus                GGGACAATCACCACTCTGGCAAAAACAGAAGGGCCAATGAAACTCTACAG
Manis pentadactyla            --------------------------------------------------

                              236 240       250       260       270       280
                              |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                  CGGGCTGCCTGCGGGGCTTCAGCGGCAAATCAGCTCCGCCTCTCTCAGGA
Bradypus variegatus           ??????????????????????????????????????????????????
Choloepus hoffmanni           ??????????????????????????????????????????????????
Mylodon darwinii              ??????????????????????????????????????????????????
Cyclopes didactylus           ??????????????????????????????????????????????????
Dasypus novemcinctus          CGAGCTGCCTGCTGGCCTGCAGAAACAGATGCACTCCACCCCT---AGGA
Procavia capensis             ---GCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCCTCTCTTAGAA
Elephas maximus               TGGGCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Mammuthus primigenius         TGGGCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Loxodonta africana            TGGGCTGCCTGCTGGCCTTCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Trichechus manatus            TGGGCTGCCTGCTGGCTTTCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Dugong dugon                  TGGGCTGCCTGCTGGCTTTTAGAGACAGATAAGCTTTGCCTCTCTTAGGA
Hydrodamalis gigas            TGGTCTGCCTGCTGGCTTTCAGAGACAGATAAGCTTTGCCTCTCTTAGGA
Sus scrofa                    AGGGCTGCCTGCTGGCCTCCAGAGACAAATAAACTTCACCTCTCTTAGGA
Sus verrucosus                AGGGCTGCCTGCTGGCCTCCAGAGACAAATAAACTTCACCTCTCTTAGGA
Sus cebifrons                 AGGGCTGCCTGCTGGCCTCCAGAGACAAATAAACTTCACCTCTCTTAGGA
Physeter microcephalus        GGGGCTGTCAGCTGGCCTCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Delphinapterus leucas         --------------------------------------------------
Lipotes vexillifer            TGGGCTGCCAGCTGGCCTCCAGAGACAAATAAGCTCTGCCTCTCTTAGGA
```

```
Balaena mysticetus             CGGGCTGCCTGCTGGCCTCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Balaenoptera acutorostrata     CGGGCTGCCTGCTGGCCCCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Balaenoptera physalus          TGGGCTGCCAGCTGGCCTCCAGAGACAAATAAGCTTTGCTTCTCTTAGGA
Balaenoptera bonaerensis       CGGGCTGCCTGCTGGCCTCCAGAGACAAATAAGCTTTGCCTCTCTTAGGA
Equus asinus                   CGGGCTGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCCTCTCTGAGGA
Equus quagga boehmi            CGGGCTGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCCTCTCTGAGGA
Equus przewalskii              CGGGCCGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCCTCTCTGAGGA
Equus caballus                 CGGGCCGCCTGCTGGCCTCCAGAGATAAATAAGCTTCGCCTCTCTGAGGA
Manis pentadactyla             --------------------------------------------------

                               286 290       300       310       320       330
                               |...|....|....|....|....|....|....|....|....|...
Homo sapiens                   TCGGCCTCTACGACACGGTCCAGGAGTTCCTCACCGCAGGGAAAGAAAgt
Bradypus variegatus            ??????????????????????????????????????????????????
Choloepus hoffmanni            ??????????????????????????????????????????????????
Mylodon darwinii               ??????????????????????????????????????????????????
Cyclopes didactylus            ??????????????????????????????????????????????????
Dasypus novemcinctus           TGGGACTCTAGGATACCGTCCCGCGGC-TTTCACCGCAGGGAAACCAGca
Procavia capensis              TTGGCTTCTGTGACA-GGTTCAGGAGTACTTCACTGGAGGAAAAGAAAgt
Elephas maximus                CTGGCCTCTATGATATGGCCCAGGAGTATTCCACTGCAGAGAAAGAAAgt
Mammuthus primigenius          CTGGCCTCTATGATATGGCCCAGGAGTATTCCACTGCAGAGAAAGAAAgt
Loxodonta africana             CTGGCCTCTATGATATGGCCCAGGAGTATTCCACTGCAGAGAAAGAAAgt
Trichechus manatus             TTGGCCTCTATGATACTGTCCAGGAGTATTTCACTGCAGGGAAAGAAAgt
Dugong dugon                   TTGGCCTCTATGATACTGTCCAGGAGTACTTCACTGCAGGGTAAGAAAgt
Hydrodamalis gigas             TTGGCCTCTATGATACTGTA?AGGAGTACTTCACTGCAGGGTAAGAAAgt
Sus scrofa                     TCAGCCTCTATGATACCATCCAGG----------------GAGAGAAAgt
Sus verrucosus                 TCACCCTCTATGATACCATCCAGG----------------GAGAGAAAgt
Sus cebifrons                  TCAGCCTCTATGATACCATCCAGG----------------GAGAGAAAgt
Physeter macrocephalus         TCAGCCTCTATGAGAGCATCCAGGAGCTCTTCACCACAGGGAAAGAAAgt
Delphinapterus leucas          --------------------------------------------------
Lipotes vexillifer             TCAGCCTCTATGAGAGCAT-CAGGAGTTCTTCACCACAGGGAAAGAAAgt
Balaena mysticetus             TCAGCATCTATGAAAGCATCCAGGAGTTCTTCACCACAGGGAAAGAAAgt
Balaenoptera acutorostrata     TCAGCCTCTATGAAAGCATCCAGGAGTTCTTCACCACAGGGAAAGAAAgt
Balaenoptera physalus          TCAGCCTCTATGAAAGCATCCAGGAGTTCTTCACCACAGGGAAAGAAAgt
Balaenoptera bonaerensis       TCAGCCTCTATGAAAGCATCCAGGAGTTCTTCACCACAGGGAAAGAAAgt
Equus asinus                   TTGGCCTCTATGATACGGTCCAGGAGTTCTTCACCACAGGGAAAGAAAgt
Equus quagga boehmi            TTGGCCTCTATGATACGGTCCAGGAGTTCTTCACCACAGGGAAAGAAAgt
Equus przewalskii              TTGGCCTCTATGATACGGTCCAGGAGTTCTTCACCACACGGAAAGAAAgt
Equus caballus                 TTGGCCTCTATGATACGGTCCAGGAGTTCTTCACCACACGGAAAGAAAgt
Manis pentadactyla             --------------------------------------------------
```

Exon 3

```
                                 334   340       350       360       370       380
                                |.....|....|....|....|....|....|....|....|....|....|
Homo sapiens                   agCAGCACCTAGTTTAGGAAGCAAGATTTTAGCTGGTCTAACGACTGGAGGAGT
Bradypus variegatus            ??????????????????????????????????????????????????????
Choloepus hoffmanni            ??????????????????????????????????????????????????????
Mylodon darwinii               ag------GTAGTTTAGAAATCAAGATTTCAGTTGGCTTAACAACTGGAGAAGT
Cyclopes didactylus            ??????????????????????????????????????????????????????
Dasypus novemcinctus           ------------------------------------------------------
Procavia capensis              ------------------------------------------------------
Elephas maximus                aaCTCCAGCTAGTTTAGAAAGCAAGATCGCTGCTGGCTTAAGGACTGGAGGAGT
Mammuthus primigenius          aaCTCCAGCTAGTTTAGAAAGCAAGATCGCTGCTGGCTTAAGGACTGGAGGAGT
Loxodonta africana             aaCTCCAGCTAGTTTAGAAAGCAAGATCGCTGCTGGCTTAAGGACTGGAGGAGT
Trichechus manatus             agCACCGGCTAGTTTAGGAAGCAAGATCTCTGCTGGCTTAACGACTGGAGGAGT
Dugong dugon                   agCACCGGCTAGTTTAGGAAGCAAGATCTCCGCTGGCTTAACGACTGGAGGAGT
Hydrodamalis gigas             agCACCGGCTAGTTTAGGAAGCAAGATCTCCGCTGGCTTAATGACTGGAGGAGT
Sus scrofa                     ------------------------------------------------------
Sus verrucosus                 ------------------------------------------------------
```

```
Sus cebifrons                 ------------------------------------------------------
Physeter macrocephalus        aacaacagCTAGTTTAGGAAGCCAGGTCTCAGCGGGCCTAACGACTGGCGGTGT
Delphinapterus leucas         ------------------------------------------------------
Lipotes vexillifer            aacaacggCTAGTTTAGTGAGAAGCAGGGTCTCAGCGGGCCTAACGACTGGCGG
Balaena mysticetus            aacaacggCTAGTTTAGGAAGCAGGGTCTCAGCGGGCCTAACGAATGGCGGCGT
Balaenoptera acutorostrata    aacaacggCTAGTTTAGGAAGCAGGGTCTCAGCGAGCCTAACGAATGGCGGCGT
Balaenoptera physalus         aacaacggCTAGTTTAGGAAGCAGGGTCTCAGCGGGCCTAACGAMTGGCGGCGT
Balaenoptera bonaerensis      aacaacggCTAGTTTAGGAAGCAGGGTCTCAGCGGGCCTAACGACTGGCAGCAT
Equus asinus                  ag------CTAGTTTAGGAAGCAAGGTCTCAGCTGGCCTAACGACTGGAGGAGT
Equus quagga boehmi           ag------CTAGTTTAGGAAGCAAGGTCTCAGCTGGCCTAACGACTGGAGGAGT
Equus przewalskii             ag------CTAGTTTAGGAAGCAAGGTCTCAGCTGGCCTAACGACTGGAGGAGT
Equus caballus                ag------CTAGTTTAGGAAGCAAGGTCTCAGCTGGCCTAACGACTGGAGGAGT
Manis pentadactyla            agCAACACTTCAGTTAGGAAGTAAGATCTCAGCTGAGCTAA-GACTAAAGGAGA

                              385 390       400       410       420       430

                              |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                  GGCAGTATTCATTGGGCAACCCACAGAGGTCGTGAAAGTCAGACTTCAAG
Bradypus variegatus           ??????????????????????????????????????????????????
Choloepus hoffmanni           ??????????????????????????????????????????????????
Mylodon darwinii              GGCCAT????????????????????????????????????????????
Cyclopes didactylus           ??????????????????????????????????????????????????
Dasypus novemcinctus          --------------------------------------------------
Procavia capensis             --------------------------------------------------
Elephas maximus               GGCAGTGTTCACT-GGCAACCCACAGAGGTCGTGGAGGCTAGACTTCAAG
Mammuthus primigenius         GGCAGTGTTCACT-GGCAACCCACAGAGGTCGTGGAGGCTAGACTTCAAG
Loxodonta africana            GGCAGTGTTCACT-GGCAACCCACAGAGGTCGTGGAGGCTAGACTTCAAG
Trichechus manatus            GGCGGTATTCATTGGGCAACCCACAGAGGTCGTGCAGGTCAGACATCAAG
Dugong dugon                  GGTGGTATTCACTGGGCAACCCACAGAGGTCATGCAGGTCAGACATCAAG
Hydrodamalis gigas            GGCGGTATTTGCTGGGCAACCCACAGAGGTCATGCAGGTCAGACATCAAG
Sus scrofa                    --------------------------------------------------
Sus verrucosus                --------------------------------------------------
Sus cebifrons                 --------------------------------------------------
Physeter microcephalus        GGCCGTGTTCATTGGGCAACGCACAGAGGTAGTGAAGGTCAGACTTCACG
Delphinapterus leucas         -----------------------------TGGTGAAGGTCAGACTTCAAG
Lipotes vexillifer            GGCCGTGTTCGTTGGGCAACCCACAGAGGTAGTGATGGTCAGACTTCACA
Balaena mysticetus            GGCCGTGTTCATTGGGCAACCCACAGAGGTAGTGAAGGTCAGACTTCAAG
Balaenoptera acutorostrata    GGCCGTGTTCATTGGGCAACCCACAGAGGTAGTGAAGGTCAGACTTCAAG
Balaenoptera physalus         GGCTGTGTTCATTGGGCAACCCACAGAGGTAGTGAAGGTCAGACTTCAAG
Balaenoptera bonaerensis      GGCCGTGTTCATTGGGCAACCCACAGAGGTAGTGAAGGTCAGACTTCAAG
Equus asinus                  GGCAGTATTCATTGGGCAACCCACCGAGGTCATGAAGGTCAGACTTCAAG
Equus quagga boehmi           GGCAGTATTCATTGGGCAACCCACYGAGGTCGTGAAGGTCAGACTTCAAG
Equus przewalskii             GGCAGTATTCATTGGGCAACCCACCGAGGTCGTGAAGGTCAGACTTCAAG
Equus caballus                GGCAGTATTCATTGGGCAACCCACCGAGGTCGTGAAGGTCAGACTTCAAG
Manis pentadactyla            GACAGTATTTATTGGGCAAC-CACAGAGGTTGTGAAGGTCAGACTTCAAG

                              436 440       450       460       470       480
                              |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                  CACAGAGCCATCTCCACGGAATCAAACCTCGCTACACGGGGACTTATAAT
Bradypus variegatus           ??????????????????????????????????????????????????
Choloepus hoffmanni           ??????????????????????????????????????????????????
Mylodon darwinii              ??????????????????????????????????????????????????
Cyclopes didactylus           ??????????????????????????????????????????????????
Dasypus novemcinctus          --------------------------------------------------
Procavia capensis             --------------------------------------------------
Elephas maximus               CACAGAGCCATCTACATGGTCTCAAACCGTGCTACACTGGGACTTACAAT
Mammuthus primigenius         CACAGAGCCATCTACATGGTCTCAAACCATGCTACACTGGGACTTACAAT
Loxodonta africana            CACAGAGCCATCTACATGGTCTCAAACCGTGCTACACTGGGACTTACAAT
Trichechus manatus            TGCAGAGCCATCTATGTGGTCTCAAACTGCGCTACACTGGGACTTACAAT
Dugong dugon                  TGCAGAGCCATCTGTGTGGTCTCAAACCGCGCTACACTGGGACTTACAAT
```

```
Hydrodamalis gigas             TGCAGAGCCATCTGTGTGGTCTCAAACCGCGCTACACTGGGACTTACAAT
Sus scrofa                     --------------------------------------------------
Sus verrucosus                 --------------------------------------------------
Sus cebifrons                  --------------------------------------------------
Physeter microcephalus         CGCCGAGCCGTCTACGTGGTCCCAAACCTCGCCACGCTGGGACTTACAAT
Delphinapterus leucas          CGCAGAGCCATCTGCATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Lipotes vexillifer             CGCTGAGCCATCTACGTGGTCCTAAACCTCACTACACTGGGACTTACGAT
Balaena mysticetus             TGCCAAGCGGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Balaenoptera acutorostrata     CGCCAAGCAGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Balaenoptera physalus          CGCCAAGCSGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Balaenoptera bonaerensis       CGCCAAGCAGTCTACATGGTCCCAAACCTCGCTACACTGGGACTTACAAT
Equus asinus                   CGCAGAGCCATCTGCATGGTCCCAAACCTCGCTACACTGGGACTTATAAT
Equus quagga boehmi            CGCAGAGCCATCTGCATGGTCCCAAACCTCGCTRCACTGGGACTTATAAT
Equus przewalskii              CGCAGAGCCATCTGCATGGTCCCAAACCGCGCTACACTGGTACTTATAAT
Equus caballus                 CGCAGAGCCATCTGCATGGTCCCAAACCGCGCTACACTGGTACTTATAAT
Manis pentadactyla             CACAGACCCATCCACATGGTGTCAAACCTCACTACATGGGGACTTAC--T

                               486 490       500       510       520       530
                               |...|....|....|....|....|....|....|....|....|....
Homo sapiens                   GCGTACAGAATAATAGCAACAACCGAAGGCTTGACGGGTCTTTGGAAAGgt
Bradypus variegatus            ???????????????????????????????????????????????????
Choloepus hoffmanni            ???????????????????????????????????????????????????
Mylodon darwinii               ???????????????????????????????????????????????????
Cyclopes didactylus            ???????????????????????????????????????????????????
Dasypus novemcinctus           ---------------------------------------------------
Procavia capensis              ---------------------------------------------------
Elephas maximus                GCTGACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGGAAAGgt
Mammuthus primigenius          GCTGACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGGAAAGgt
Loxodonta africana             GCTGACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGGAAAGgt
Trichechus manatus             GCTTACAGAATCATAGCAACAACAGAACGCTTGACAGGTCTTTGAAAAGgt
Dugong dugon                   GCTTACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGAAAAGgt
Hydrodamalis gigas             GCTTACAGAATCATAGCAACAACAGAAGGCTTGACAGGTCTTTGAAAAGgt
Sus scrofa                     ---------------------------------------------------
Sus verrucosus                 ---------------------------------------------------
Sus cebifrons                  ---------------------------------------------------
Physeter microcephalus         GCTCACAGAATTATAGCAACAACAGAAGGCTTGATGGGGCTTTGGAAAGat
Delphinapterus leucas          GCTTACAGAATTATAGCAACAACAGAAGGCTTGACGGGGCTTTGGAAAGgt
Lipotes vexillifer             GCTCACAGAATTATAGCAACAACAGAAAGCTTGACGGGGCTTTGGAAAGgt
Balaena mysticetus             GCTTATAGAATTATAGCAACAACAGAAGGCTTGACGGGGCTTTGGAAAGgt
Balaenoptera acutorostrata     GCTTACAGAATTATAGCAACAACAGAAGGCTTGATGGGGCTTTGGAAAGgt
Balaenoptera physalus          GCTTACAGAATTATAGCAACAACAGAAGGCTTGACGGGGCTTTGGAAAGgt
Balaenoptera bonaerensis       GCTTACAGAATTATAGCAACAACAGAAGGCTTGATGGGGCTTTGGAAAGgt
Equus asinus                   GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Equus quagga boehmi            GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Equus przewalskii              GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Equus caballus                 GCCTACAGAATTACAGCAACAACAGAAGGCTGGACGGGTCTTTGGAAAGgt
Manis pentadactyla             GCTGACAGAATTAGAGCAACAACTGAAGGCTTGACAGATCTTTGGAAAGct
```

Exon 4

```
                               535  540       550       560       570       580
                               |....|....|....|....|....|....|....|....|....|....|
Homo sapiens                   agGGACTACTCCCAATCTGATGAGAAGTGTCATCATCAATTGTACAGAGCTAG
Bradypus variegatus            ?????????????????????????????????????????????????????
Choloepus hoffmanni            ?????????????????????????????????????????????????????
Mylodon darwinii               ?????????????????????????????????????????????????????
Cyclopes didactylus            ?????????????????????????????????????????????????????
Dasypus novemcinctus           -----------------------------------------------------
Procavia capensis              -----------------------------------------------------
Elephas maximus                agGGACTACTCTCAGTCTGACAAGAAATATCATCATCAGTTGTACAGAGCTAG
```

```
Mammuthus primigenius          agGGACTACTCTCAGTCTGACAAGAAATATCATCATCAGTTGTACAGAGCTAG
Loxodonta africana             agGGACTACTCTCAATCTGACAAGAAATATCATCATCAGTTGTACAGAGCTAG
Trichechus manatus             agGGATTACTCCCAATCTGACAAGAAATGTCATCATCATTTGTACAGAGCTAG
Dugong dugon                   agGGACTACTCCCAATCTGACAAGAAATGTCATCATCAATTGTACAGAGCTAG
Hydrodamalis gigas             agGGACTACTCCCAATCTGACAAGAAATGTCATCATCAATTGTACAGAGCTAG
Sus scrofa                     -----------------------------------------------------
Sus verrucosus                 -----------------------------------------------------
Sus cebifrons                  -----------------------------------------------------
Physeter macrocephalus         agGTTCTACTCCAAATCTGACAAGAATTGTCATCATCGGCTGTACAGAGCTAG
Delphinapterus leucas          tcACTTTATATAGTGCTTCAGCAATTTACTACCGATTTGCTTTCTAGTATGTC
Lipotes vexillifer             agGTTCTACTCCAAATCTGACAAGAACTGTCATCATCAGCTGTACAGAGCTAG
Balaena mysticetus             agGTTCTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaenoptera acutorostrata     agGTTCTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaenoptera physalus          agGTTCTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTACAGAGCTAG
Balaenoptera bonaerensis       agGTTCTACTCCGAATCTGACAAGAATTGTCATCATCAGCTGTATAGAGCTAG
Equus asinus                   agGGACCACTCTCAATCTGACAAGAAATGTCATCATCAATTGTACAGAGCTAG
Equus quagga boehmi            agGGACCACTCTCAATCTGACAAGAAATGTCATCATCAATTGTACAGAGCTAG
Equus przewalskii              agGGACCACTCTCAATCTGACAAGAAATGTCATCATCAATTGTACAGAGCTAG
Equus caballus                 agGGACCACTCTCAATCTGACAAGAAATGTCATCATCAATTGTACAGAGCTAG
Manis pentadactyla             atGGTCTACTCCCAATCTGATGAGAAATGTCATCATGAATTGTGCAGAGCTAG

                               586  590       600       610       620       630
                               |....|....|....|....|....|....|....|....|....|....|
Homo sapiens                   TAACATATGATCTAATGAAGGAGGCCTTTGTGAAAAACAACATATTAGCAGgt
Bradypus variegatus            ?????????????????????????????????????????????????????
Choloepus hoffmanni            ?????????????????????????????????????????????????????
Mylodon darwinii               ?????????????????????????????????????????????????????
Cyclopes didactylus            ?????????????????????????????????????????????????????
Dasypus novemcinctus           -----------------------------------------------------
Procavia capensis              -----------------------------------------------------
Elephas maximus                TAACATATGACCTGATGAAGGAGACCTTTGTGAAAAACAACCTACTAGCAGct
Mammuthus primigenius          TAACATATGACCTGATGAAGGAGACCTTTGTGAAAAACAACCTACTAGCAGct
Loxodonta africana             TAACATATGACCTGATGAAGGAGACCTTTGTGAAAAACAACCTACTAGCAGct
Trichechus manatus             TAACACATGACCTAATGAAGGAGACCTTTGTGAAAAACAATCTACTGGCAGgt
Dugong dugon                   TAACACATGACCTAATGAAGGAGACCTTTGTGAAAAACAATCTACTGGCAGgt
Hydrodamalis gigas             TAACACATGACCTAATGAAGGAGACCTTTGTGAAAAACAATCTACTGGCAGat
Sus scrofa                     -----------------------------------------------------
Sus verrucosus                 -----------------------------------------------------
Sus cebifrons                  -----------------------------------------------------
Physeter macrocephalus         TAACATATGACCTAATGAAGGAGGCCCTTGTGAAAAACA----ATTAACAGgt
Delphinapterus leucas          ACTATCTCATTTGACATGCTCCTGCCCTTGTGAAAAACA----ATTAACAGgt
Lipotes vexillifer             TAACATATGACCTAATGAAGGAGACCCTTGTGAAAAACA----ATTAACAGgt
Balaena mysticetus             TAACGTATGACCTAATGAAGGAGACCCTTGTGAAAAACA----ATTAACAGgt
Balaenoptera acutorostrata     TAACGTATGACCTAATGAAGGAGGCCCTTGTGAAAAACA----ATTAACAGgt
Balaenoptera physalus          TAACGTATGTCCTAATGAAGGAGGCCCTTGTGAAAAACA----ATTAACAGgt
Balaenoptera bonaerensis       TAACGTATGACCTAATGAAGGAGGCCCTTGTGAAAAACA----ATTAACAGgt
Equus asinus                   TAACCCATGACCTAATGAAGGAGGCCCTTGTGAAAAACCAACTATTAGCAGgt
Equus quagga boehmi            TAACCCATGACCTAATGAAGGAGGCCCTTGTGAAAAACCAACTATTAGCAGgt
Equus przewalskii              TAACCCATGACCTAATGAAGGAGGCCCTTGTGAAAAACCAACTATTAGCAGgt
Equus caballus                 TAACCCATGACCTAATGAAGGAGGCCCTTGTGAAAAACCAACTATTAGCAGgt
Manis pentadactyla             TAA-GTATGACCTAATGAAGGAGGCACCTCTAAACAAGA----CTTACCAGct
```

Exon 5

```
                                 636 640       650       660       670       680
                                 |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                   agATGACGTCCCCTGCCACTTGGTGTCGGCTCTTATCGCTGGATTTTGC---
```

```
Bradypus variegatus           ????????????????????????????????????????????????????
Choloepus hoffmanni           ????????????????????????????????????????????????????
Mylodon darwinii              ????????????????????????????????????????????????????
Cyclopes didactylus           ????????????????????????????????????????????????????
Dasypus novemcinctus          ----------------------------------------------------
Procavia capensis             agGTCATGTGCCCTGCCACTTAGTGTTTGCTCTTATTGTTGGATTTTGC---
Elephas maximus               agATGACGTGCCCTGCCACTTAGTGTCTTCTCTTATCGCTGGATTTTGC---
Mammuthus primigenius         agATGACGTGCCCTGCCACTTAGTGTCTTCTCTTATCGCTGGATTTTGC---
Loxodonta africana            agATGACGTGCCCTGCCACTTAGTGTCTGCTCTTATCGCTGGATTTTGC---
Trichechus manatus            agATGATGTGCCTTGCCACTTAGTGTCTGCTCTCATTGCTGGATTTTGC---
Dugong dugon                  agATGATGTGCCCTGCCACTCGGTGTCTGCTCTCATTGCTGGATTCTGC---
Hydrodamalis gigas            agATGATGTGCCCTGCCACTCAGTGTCTGCTCTCATTGCTGGATTTTGC---
Sus scrofa                    ----------------------------------------------------
Sus verrucosus                ----------------------------------------------------
Sus cebifrons                 ----------------------------------------------------
Physeter macrocephalus        agATGATGTGCCCTGTCACTTTGTGTCCACTGTTATCGCTGGATTTTGC---
Delphinapterus leucas         agATGATGTGCCCGGTCACTTTGTGTCCGCTGTTATTGCTGGATTTTGC---
Lipotes vexillifer            agACGATGTGCCCGGTCACTTTGTGTCCGCTGTTATCGCTGGATTTTGC---
Balaena mysticetus            agATGATGTGCCCTGTCACTTTGTGTCCGCTGTTATCGCTGGATTTTGC---
Balaenoptera acutorostrata    agATGATGTACCCTGTCACTTTGTGTCCGCTGTTATCGCTGGATTTTGC---
Balaenoptera physalus         agATGATGTGCCCTGTCACTTTGTGTCCGCTGTTATCGCTGGATTTTGC---
Balaenoptera bonaerensis      agATGATGTGCCCTGTCACTTTGTGTCCGCTGTTATCGCTGGATTTTGC---
Equus asinus                  agATGATGTACCTTGCCACTTTGTGCCGGCTGTCATCGCCAGATTTTGT---
Equus quagga boehmi           agATGATGTACCTTGCCACTTTGTGTCGGCTGTCATCGCCAGATTTTGT---
Equus przewalskii             agATGATGTACCTTGCCACTTTGTGTCGGCTGTCATCGCCAGATTTTGT---
Equus caballus                agATGATGTACCTTGCCACTTTGTGTCGGCTGTCATCGCCAGATTTTGT---
Manis pentadactyla            agATGATGTACC-TGCCACTTTGTATCAGCTCTTATCACCTGATTTTGCACA

                              686 690       700       710       720       730
                              |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                  -GCAACAGCTATGTCCTCCCCGGTGGATGTAGTAAAAACCAGATTTATTA
Bradypus variegatus           ??????????????????????????????????????????????????
Choloepus hoffmanni           ??????????????????????????????????????????????????
Mylodon darwinii              ??????????????????????????????????????????????????
Cyclopes didactylus           ??????????????????????????????????????????????????
Dasypus novemcinctus          --------------------------------------------------
Procavia capensis             -ACAATTGTTCTGTCCTCTCCACTGGACATGGTGAAAACCAGATTTGTGA
Elephas maximus               -ACAATGGTTCTGTCCTCTCCAGCAGATGTGGTGAAAACCAGATTTATTA
Mammuthus primigenius         -ACAATGGTTCTGTCCTCTCCAGCAGATGTGGTGAAAACCAGATTTATTA
Loxodonta africana            -ACAATGGTTCTGTCCTCTCCAGCAGATGTGGTGAAAACCAGATTTATTA
Trichechus manatus            -ACAACAGTTCTGTTCTCTCCAGTGGATGTGGTGAAAACCAGATTTGTTA
Dugong dugon                  -ACAACAGTTCCGTCCTCTCCAGTGGATGTGGTGAAAACCAGATTTGTTA
Hydrodamalis gigas            -ACAACAGTTCTGTCCTCTCCAGTGGATGTGGTGAAAACCAGATTTGTTA
Sus scrofa                    --------------------------------------------------
Sus verrucosus                --------------------------------------------------
Sus cebifrons                 --------------------------------------------------
Physeter microcephalus        -ACAACGGTTCTGTCCTCTCCAGTGGATGCGGTGAAAACCAGATTTGTTA
Delphinapterus leucas         -ACAACGGTTCTGTCCTCTCCAGTGGATGTGGTGAAAACCAGATTTGTTA
Lipotes vexillifer            -ACAACGGTTCTGTCCTCTCCAGTGGATGTGGTGAAAACCAGATTTGTTA
Balaena mysticetus            -ACAACGGTTCTGTCCTCTCCAGTGGATGTGGTGAAAACCAGATCTGTTA
Balaenoptera acutorostrata    -ACAACGGTTCTGTCCTCTCCAGTGGATGTGGTGAAAACCAGGTCTGTTA
Balaenoptera physalus         -ACAACGGTTCTGTCCTCTCTAGTGGATGTGGTGAAAACCAGATTTGTTA
Balaenoptera bonaerensis      -ACAACGGTTCTGTCCTCTCCAGTGGATGTGGTGAAAACCAGGTCTGTTA
Equus asinus                  -GCAACAGTTCTGTCTTCTCCAGTGGACATGGTGAAAACCACATTTGTTA
Equus quagga boehmi           -GCAACAGTTCTGTCTTCTCCAGTGGACATGGTGAAAACCACATTTGTTA
Equus przewalskii             -GCAACAGTTCTGTCTTCTCCAGTGGACATGGTGAAAACCACATTTGTTA
Equus caballus                -GCAACAGTTCTGTCTTCTCCAGTGGACATGGTGAAAACCACATTTGTTA
Manis pentadactyla            AACAATGTTTTTGTCC-CTCTGGTGGATATGGTGAAAAGCAGATTTGTTA

                              736 740       750       760       770       780
```

```
                              |...|....|....|....|....|....|....|....|....|....|
Homo sapiens                  ATTCTCCACCAGGACAG---TACAAAAGTGTGCCCAACTGTGCAATGAAA
Bradypus variegatus           ??????????????????????????????????????????????????
Choloepus hoffmanni           ??????????????????????????????????????????????????
Mylodon darwinii              ??????????????????????????????????????????????????
Cyclopes didactylus           ??????????????????????????????????????????????????
Dasypus novemcinctus          --------------------------------------------------
Procavia capensis             ATTCTCCACCAAGATAC---TAAACTAGTGTACCCAAACATGCAACCATC
Elephas maximus               ATTCTCCACCAGGATAC---TACACGAGTGTACCCAAATGCGCAGTGACC
Mammuthus primigenius         ATTCTCCACCAGGATAC---TACACGAGTGTACCCAAATGCGCAGTGACC
Loxodonta africana            ATTCTCCACCAGGATAC---TACACGAGTGTACCCAAATGCGCAGTGACC
Trichechus manatus            ATTCTCCACCAGGATAC---TACACAAGCATACCCAAATGTGCAATGACC
Dugong dugon                  ATTCTCCACCAGGATAC---TACACAAGCATGCCCAAATGTGCAATGACC
Hydrodamalis gigas            ATTCTCCACCAGGATAC---TACACAAGCATACCCAAATGTGCAATGACC
Sus scrofa                    --------------------------------------------------
Sus verrucosus                --------------------------------------------------
Sus cebifrons                 --------------------------------------------------
Physeter microcephalus        ACTGTCTACCGGGACAG---TACACAAGTGTGTCCAACTGTGCAATGATG
Delphinapterus leucas         ACTGTCTACCGGGACAG---TACACAAGTGTGTCCAACTGCGCAATGATG
Lipotes vexillifer            ACTGTCTACCGAGACAG---TACATAAGCGTGTCCAACTGCGCAATGATG
Balaena mysticetus            ACTGTCTACCGGGACGGACACACACAAGTGTGTCCGACTGCGCAATGATG
Balaenoptera acutorostrata    ACTGTCTACCGGGACGG---TACACAAGTGTGTCCGACTGCGCAATGTTG
Balaenoptera physalus         ACTGTCTACCRGGACGG---TACACAAGTGTGTCCGACTGCGCAATGTTG
Balaenoptera bonaerensis      ACTGTCTACCGGGACGG---TACACAAGTGTGTCCGACTGCGCAATGTTG
Equus asinus                  ATTCTCCACCAGGACAG---TACACAAGTGTGCCCAACTGTGCAACGACA
Equus quagga boehmi           ATTCTCCACCAGGACAG---TACACAAGTGTGCCCAACTGTGCAACGACA
Equus przewalskii             ATTCTCCACCAGGACAG---TACACAAGTGTGCCCAACTGTGCAACGACA
Equus caballus                ATTCTCCACCAGGACAG---TACACAAGTGTGCCCAACTGTGCAACGACA
Manis pentadactyla            ATTCTCTACCAGGACAA---TGCACAAGTGTGCCCACCTGTGCAATGACA

                              786 790       800       810       820
                              |...|....|....|....|....|....|....|....
Homo sapiens                  GTGTTCACTAACGAAGGACCAACGGC-TTTCTTCAAGGGgt
Bradypus variegatus           ?????????????????????????????????????????
Choloepus hoffmanni           ?????????????????????????????????????????
Mylodon darwinii              ?????????????????????????????????????????
Cyclopes didactylus           ?????????????????????????????????????????
Dasypus novemcinctus          -----------------------------------------
Procavia capensis             ATGTTTACCAAGAAAAGACCACCAGC-TTTTTTCAAAGGgt
Elephas maximus               ATGTTCACCAAGAAAGGACTGCCGGC-TTTTTTCAACGGgt
Mammuthus primigenius         ATGTTCACCAAGAAAGGACTGCCGGC-TTTTTTCAACGGgt
Loxodonta africana            ATGTTCACCAAGAAAGGACTGCCGGC-TTTTTTCAACGGgt
Trichechus manatus            GTGTTCACCAAGGAAGGACTGCCGGC-TTTTTTCAAAGGgt
Dugong dugon                  GCGTTCACC-AGGAAGGACTGCCGGCTTTTTTTCAAAGGgt
Hydrodamalis gigas            GTGTTCACCAGGGAAGGACTGCCGGC-TTTTTTCAAAGGgt
Sus scrofa                    -----------------------------------------
Sus verrucosus                -----------------------------------------
Sus cebifrons                 -----------------------------------------
Physeter microcephalus        GTCCTTCCTAAGGAAGGACCATCGGC-TTTTTTCAAAGGgt
Delphinapterus leucas         ATGCCCACTAAGGAAGGACCATCGGC-TTTTTTCAAAGGgt
Lipotes vexillifer            ATGCTCACTAAGGAAGGACCATCGGC-TTTTTTCAAAGGgt
Balaena mysticetus            ATGCTCACTAAGGAAGGACCATCGGC-TTTTTTCAAAGGgt
Balaenoptera acutorostrata    ATGCTCACTAAGGAAGGACCATCGGC-TTTTTTCAAAGGgt
Balaenoptera physalus         ATGCTCACTAAGGAAGGACCATCGGC-TTTTTTCAAAGGgt
Balaenoptera bonaerensis      ATGCTCACTAAGGAAGGACCATCGGC-TTCTTTCAAAGGgt
Equus asinus                  ATGCTCACTAAGGAAGGACCGTCAGC-TTTTTTCAAAGGgt
Equus quagga boehmi           ATGCTCACTAAGGAAGGACCGTCAGC-TTTTTTCAAAGGgt
Equus przewalskii             ATGCTCACTAAGGAAGGACCGTCAGC-TTTTTTCAAAGGgt
Equus caballus                ATGCTCACTAAGGAAGGACCGTCAGC-TTTTTTCAAAGGgt
Manis pentadactyla            GTGCTCACTAAGGAAGGACCATTGGC-TTTTTTCAAAAGgc
```

Exon 6

```
                                825  830       840       850       860       870
                                |....|....|....|....|....|....|....|....|....|....|
Homo sapiens                  agGTTGGTACCTTCCTTCTTGCGACTTGGATCCTGGAACGTCATTATGTTTGT
Bradypus variegatus           agGTTTGTGCCTTCCTTCTTGCGACTTAGATCCTGGAATGTCATCATGTTTGA
Choloepus hoffmanni           agGTTTGTGCCTTTCTTCTTGAGACTTGGATCCTGAAATGTCATCATGTTTGT
Mylodon darwinii              ????????????????????????????????????????????????????
Cyclopes didactylus           agATTTGTACCTTCCTTCTTGCAACTAGGATCCTGGAATGTCATCATGTTTGT
Dasypus novemcinctus          agTCTTATGCCTTCCTTCTTGTGACTCAGATCCTGGAATGTTGTCATGTTTGT
Procavia capensis             agGTTTGTACCTTTCCTCTTGTGACTAAGACTGGGAAGCATCATTAAGTTCAT
Elephas maximus               agGCTTGTGCCTTCCTTCTTGCGACTGGGATCCTGGAACGCCATTATGTTTTT
Mammuthus primigenius         agGCTTGTGCCTTCCTTCTTGCGACTGGGATCCTGGAACGCCATTATGTTTTT
Loxodonta africana            agGCTTGTGCCTTCCTTCTTGCGACTGGGATCCTGGAACGCCATTATGTTTTT
Trichechus manatus            agGTTTGTGCCTTCCTTCTTGCGACTCGGTTCCTGGAATGTCATTATGTT--T
Dugong dugon                  agGTTTGTGCCTTCCTTCTTGTGACTCGGATCCTGGAATGTCATTATGTT--T
Hydrodamalis gigas            agGTTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGAATGTCATTATGTT--T
Sus scrofa                    agATTTGTACCTTCCTTCTGGTGACTGGGATCCTGGAACGTCATCCCATTTGT
Sus verrucosus                agATTTGTACCTTCCTTCTGGTGACTGGGATCCTGGAACGTCATCCCATTTGT
Sus cebifrons                 agATTTGTACCTTCCTTCTGGTGACTGGGATCCTGGAACGTCATCCCATTTGT
Physeter microcephalus        agATTTGTACCTTCCTTCTAGTGACTGGGATCCTGGAACATCATCGTGT--GT
Delphinapterus leucas         agATTTGTACCTTCCTTCTTGTGACTGGGGTCCTGGAACATCATCGTGT--GT
Lipotes vexillifer            agATTTGTACCTTCCTTCTTGTGACTGGGATCCTGGAACATCATCGTGT--GT
Balaena mysticetus            agATTTGTACCTTCCTTCTTGTGACTGGGATCCTGGAACATCATCGTGT--GT
Balaenoptera acutorostrata    agATTTGTACCTTCCTTCTTGTGACTGGGATCCTGGAACATCATCGTGT--GT
Balaenoptera physalus         agATTTGTACCTTCCTTCTTGTGACTGGGATCCTGGAACATCATCGTGT--GT
Balaenoptera bonaerensis      ????????????????????????????????????????????????????
Equus asinus                  agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Equus quagga boehmi           agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Equus przewalskii             agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Equus caballus                agATTTGTGCCTTCCTTCTTGCGACTCGGATCCTGGCATGTCATCATGTTTGT
Manis pentadactyla            agATTTG----TCCCTTCTTGCACCTTGGGTCTCCAGACGTCATTATATTTGT

                              876  880       890       900       910       920
                              |....|....|....|....|....|....|....|....|....|....|
Homo sapiens                  GTGCTTTGAACAACTGAAACGAGAACTGTCAAAGTCAAGGCAGACTATGGA
Bradypus variegatus           GTA--CTGAACAGCTAAAACAGGAAAGGATGACATCAAGGCGGACTATGGA
Choloepus hoffmanni           GTG--TTGAACAGCTAAAACAGGAAATGATGACATCAAGGCA-ACTATGAA
Mylodon darwinii              ???????????????????????????????????????????????????
Cyclopes didactylus           GTG--TTGAACAGCTAAAACAAGAACTGATGACATCAAGGCAGACTATGGA
Dasypus novemcinctus          GTACTTTGAACAGCTGAAACAAGAATTGATGAAGCCAAGGCAGACTATGGA
Procavia capensis             GTGCTTTGAACAGCTGAAAAAAGAATTGATGAAGTCGAGGCAGATGATGGA
Elephas maximus               GTGCTCTGAACAGCTGAAACAAGAATTGATGAAGTCAAGGCAGACTATGGG
Mammuthus primigenius         GTGCTCTGAACAGCTGAAACAAGAATTGATGAAGTCAAGGCAGACTATGGG
Loxodonta africana            GTGCTCTGAACAGCTGAAACAAGAATTGATGAAGTCAAGGCAGACTATGGG
Trichechus manatus            GTGCTTTGAATAGCTGAAACGAGAATTGATGAAGTCGAGGGAGACTATGGA
Dugong dugon                  GTGCTTTGAACAGCTGAAACGAGAATTGATGAAGTCGAGGCAGACTATGGA
Hydrodamalis gigas            GTGCTTTGAACAGCTGAAACGAGAACTGATGAAGTCGAGGCAGACTATGGA
Sus scrofa                    GTGCTTTGAACAGCTGAAACAAGAGTTGATGGAGTCACGGCAGACTGTGGA
Sus verrucosus                GTGCTTTGAACAGCTGAAACAAGAGTTGATGGAGTCACGGCAGACTGTGGA
Sus cebifrons                 GTGCTTTGAACAGCTGAAACAAGAGTTGATGGAGTCACGGCAGACTGTGGA
Physeter microcephalus        GTGCCTTGAACAGCTGAAGCGAGAACTGCTGAAGTCGAGGCAGACCATGGA
Delphinapterus leucas         GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTTGAGGCAGACCATGGA
Lipotes vexillifer            GTGCTTTGAACAGCTGAAGTGAGAATTGATGAAGTTGAGGCAGACCATGGA
Balaena mysticetus            GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCGAGGCAGACCACGGA
Balaenoptera acutorostrata    GTGCTCTGAACAGCTGCAGCGAGAATTGATGAAGTCGAGGCAGACCATGGA
Balaenoptera physalus         GTGCTTTGAACAGCTGAAGMGAGAATTGATGAAGTCGAGGCAGACCATGGA
Balaenoptera bonaerensis      ???????????????????????????????????????????????????
Equus asinus                  GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGCAGACCAGGGA
```

```
Equus quagga boehmi           GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGCAGACCATGGA
Equus przewalskii             GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGCAGACCATGGA
Equus caballus                GTGCTTTGAACAGCTGAAGCGAGAATTGATGAAGTCAACGTGACAGACCAT
Manis pentadactyla            GTGCTCTGAACAAGGAAAATGAAAATTGACGAACTCAAGG-----------

                              925  930       940       950
                              |....|....|....|....|....|....|
Homo sapiens                  CTGTGCCACA------------------TAA
Bradypus variegatus           CTGTGCCACAGCTTCAGGAAAAGAATG-TAA
Cyclopes didactylus           CTTTGCCACAACTTCAGGAAAAGGATG-TTA
Choloepus hoffmanni           CTGTGTCACAGCTTCAGGAAAAGAATGTTAA
Mylodon darwinii              ???????????????????????????????
Dasypus novemcinctus          CTGTGCTACA------------------TAA
Procavia capensis             CTGTGCCACA------------------TAA
Elephas maximus               CTGTACCACA------------------TAA
Mammuthus primigenius         CTGTACCACA------------------TAA
Loxodonta africana            CTGTACCACA------------------TAA
Trichechus manatus            CTGTGCCACA------------------TAA
Dugong dugon                  CTGTGCCACA------------------TAA
Hydrodamalis gigas            CTGTGCCACA------------------TAA
Sus scrofa                    CTGTGCTGCA------------------TAA
Sus verrucosus                CTGTGCTGCA------------------TAA
Sus cebifrons                 CTGTGCTGCA------------------TAA
Physeter microcephalus        CTGTGCTACC------------------TAA
Delphinapterus leucas         CTGTGCTACC------------------TAA
Lipotes vexillifer            CTGTGCTACC------------------TAA
Balaena mysticetus            CTGTGCTACC------------------TAA
Balaenoptera acutorostrata    CTGTGCTACC------------------TAA
Balaenoptera physalus         CTGTGCTACC------------------TAA
Balaenoptera bonaerensis      ???????????????????????????????
Equus asinus                  CTGTGCCACA------------------TAA
Equus quagga boehmi           CTGTGCCACA------------------TAA
Equus przewalskii             CTGTGCCACA------------------TAA
Equus caballus                CTGTGCCACA------------------TAA
Manis pentadactyla            CTGTGTCATA------------------TAA
```

fig. S2. Exon alignments depicting deleterious mutations found in *UCP1* sequences of placental mammal taxa. The intact human *ucp1* sequence is provided for reference. Mutated initiation codons (green), premature stop codons (red), frameshift insertions (turquoise), deletions (dashes), frameshift deletions (pink), mutated splice sites (yellow), and mutated stop codons (teal) are indicated. Note insert from ~800 bp upstream of *ucp1* in exon 4 of beluga (grey). Missing data are denoted by question marks.

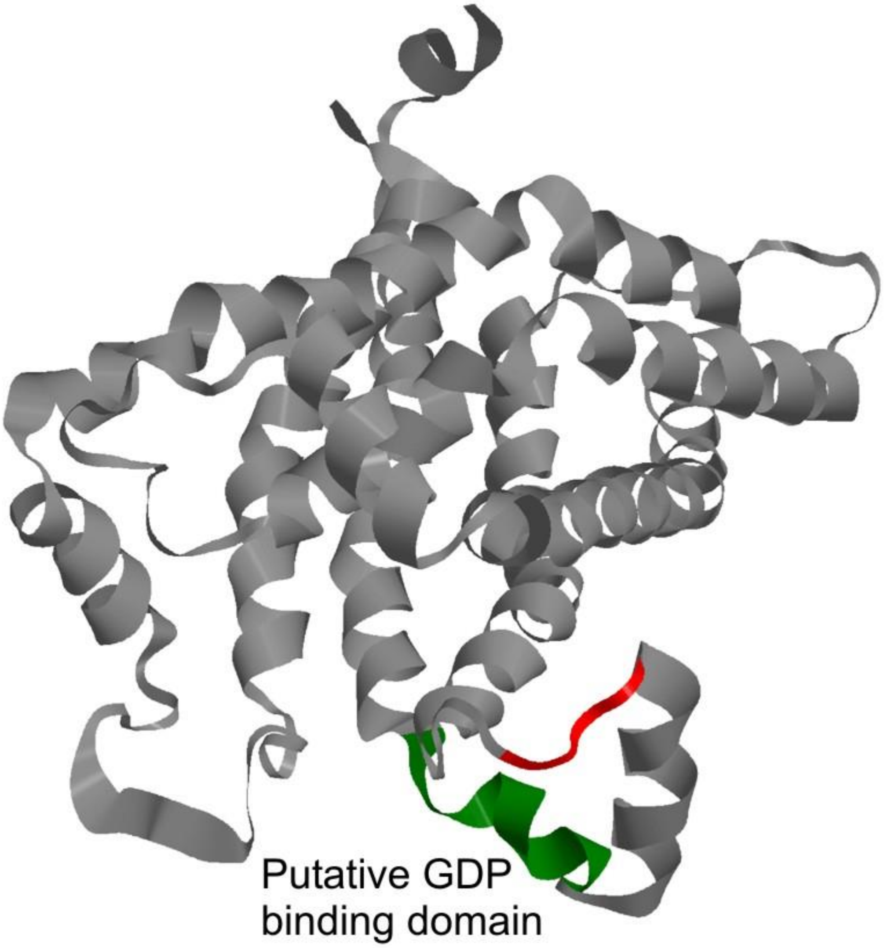

fig. S3. Ribbon diagram of residues 13 to 304 of human UCP1 (UniProt accession number P25874) structurally modelled by SWISS-MODEL. Residues highlighted in red denote the position of a four amino acid deletion in the third matrix loop of the camel (*Camelus ferus*) UCP1 protein which borders the putative GDP-binding domain (green).

		1									10										20										30		
Consensus		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Lepisosteus oculatus		M	D	R	S	V	Q	E	L	F	L	N	F	M	I	V	L	I	T	V	L	L	M	W	L	L	V	K	S	Y	Q	E	
Takifugu rubripes		M	E	R	S	A	R	E	L	F	L	N	F	M	I	V	L	I	T	V	L	L	M	W	L	L	V	K	T	Y	Q	D	
Xiphophorus maculatus		M	D	R	S	A	Q	E	L	F	L	N	F	M	I	V	L	I	T	V	L	L	M	W	L	L	V	K	T	Y	Q	D	
Danio rerio	M	M	E	R	S	T	Q	E	L	F	L	N	F	M	I	V	L	I	T	V	L	L	M	W	L	L	V	K	Q	Y	Q	D	S
Latimeria chalumnae		M	E	R	S	A	Q	E	L	F	L	N	F	M	I	V	L	I	T	V	L	L	M	W	L	L	V	K	S	Y	Q	E	
Xenopus tropicalis 1		M	E	R	S	T	Q	E	L	F	L	N	F	M	V	V	L	I	T	V	L	L	M	W	V	I	V	K	S	Y	Q	S	
Xenopus tropicalis 2		M	E	R	S	T	Q	E	L	F	L	N	F	M	I	V	L	I	T	V	L	L	M	W	L	L	V	K	S	Y	Q	E	
Ornithorhynchus anatinus		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	I	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Monodelphis domestica 1		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Monodelphis domestica 2		M	E	R	S	T	R	E	L	F	L	S	F	I	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	R	Y	
Macropus eugenii		M	E	R	S	G	R	E	L	C	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Sarcophilus harrisii		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Dasypus novemcinctus		M	E	W	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Bradypus variegatus		L	D	R	S	T	Q	D	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Choloepus hoffmanni		L	E	R	S	T	Q	D	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Mylodon darwinii		?	?	?	?	?	?	?	?	?	?	?	?	?	V	V	L	I	T	V	L	L	M	W	L	?	?	?	?	?	?	?	
Orycteropus afer		M	G	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Elephantulus edwardii		M	E	R	S	T	Q	E	L	F	L	N	F	T	I	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Echinops telfairi		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Chrysochloris asiatica		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Procavia capensis		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Trichechus manatus		M	E	R	S	T	R	E	L	C	L	N	F	A	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Hydrodamalis gigas		M	E	R	S	T	R	D	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Dugong dugon		M	E	R	S	T	R	D	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Loxodonta africana		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Mammuthus primigenius		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Elephas maximus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Oryctolagus cuniculus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Ochotona princeps		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	I	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Spermophilus tridecemlineatus		M	E	L	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Marmota marmota		M	E	L	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Fukomys damarensis		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	I	W	L	L	V	R	S	Y	Q	Y	
Heterocephalus glaber		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	I	W	L	L	V	R	S	Y	Q	Y	
Cavia aperea		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	I	W	L	L	V	R	S	Y	Q	Y	
Cavia porcellus		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	I	W	L	L	V	R	S	Y	Q	Y	
Octodon degus		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	I	W	L	L	V	R	S	Y	Q	Y	
Chinchilla lanigera	M	V	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	I	W	L	L	V	R	S	Y	Q	Y	
Dipodomys ordii		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Jaculus jaculus		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Nannospalax galili		M	E	W	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Apodemus sylvaticus		M	E	R	S	T	Q	E	L	F	I	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Mus musculus		M	E	R	S	T	Q	E	L	F	I	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Rattus norvegicus		M	E	R	S	T	Q	E	L	F	I	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Myodes glareolus		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Peromyscus maniculatus		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Microtus ochrogaster		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Microtus agrestis		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Cricetulus griseus		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Mesocricetus auratus		M	E	R	S	T	Q	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Galeopterus variegatus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	S	
Tupaia chinensis		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Otolemur garnettii		M	E	W	S	S	R	E	L	F	I	N	F	T	I	V	L	V	T	V	V	L	M	W	L	L	V	R	S	Y	Q	Y	
Propithecus coquereli		M	E	W	S	S	R	E	L	F	I	N	F	T	I	V	L	I	T	V	I	I	M	W	L	L	V	R	S	Y	Q	Y	
Daubentonia madagascariensis		M	E	W	S	T	R	E	L	F	I	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Eulemur macacaco		M	E	W	S	S	R	E	L	F	I	N	F	T	I	V	L	I	T	V	I	I	M	W	L	L	V	R	S	Y	Q	Y	
Eulemur flavifrons		M	E	W	S	S	R	E	L	F	I	N	F	T	I	V	L	I	T	V	I	I	M	W	L	L	V	R	S	Y	Q	Y	
Microcebus murinus		M	E	W	S	T	R	E	L	F	I	N	F	T	I	V	L	V	T	V	I	I	M	W	L	L	V	R	S	Y	Q	Y	
Tarsius syrichta		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Callithrix jacchus		M	E	V	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Aotus nancymaae		M	E	L	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	

Saimiri boliviensis		M	E	L	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Nomascus leucogenys		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Colobus angolensis		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Mandrillus leucophaeus		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Cercocebus atys		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Pongo abelii		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Gorilla gorilla		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Homo sapiens		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Pan troglodytes		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Pan paniscus		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Nasalis larvatus		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Rhinopithecus roxellana		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Chlorocebus sabaeus		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Macaca fascicularis		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Macaca mulatta		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Macaca nemestrina		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Papio anubis		M	G	I	N	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Condylura cristata		M	E	L	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Erinaceus europaeus		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	V	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Sorex araneus		M	E	R	S	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Eptesicus fuscus		M	D	L	S	A	R	E	L	C	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	S	
Myotis lucifugus		M	D	L	S	A	R	E	L	C	L	N	F	T	V	V	L	V	T	V	I	L	M	W	L	L	V	R	S	Y	Q	S	
Myotis brandtii		M	D	L	S	A	R	E	L	C	L	N	F	T	V	V	L	V	T	V	I	L	M	W	L	L	V	R	S	Y	Q	S	
Myotis davidii		M	D	L	S	A	R	E	L	C	L	N	F	T	V	V	L	V	T	V	I	L	M	W	L	L	V	R	S	Y	Q	S	
Rhinolophus ferrumequinum		M	E	H	S	T	R	E	L	C	L	N	F	T	I	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	S	
Megaderma lyra		M	E	R	S	T	R	D	L	C	L	N	F	T	V	V	L	I	T	V	M	L	I	W	L	L	V	R	S	Y	Q	S	
Eidolon helvum		M	E	L	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Pteropus vampyrus		M	E	L	S	T	R	E	L	C	L	N	F	T	I	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Pteropus alecto		M	E	L	S	T	R	E	L	C	L	N	F	T	I	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	Y	
Manis pentadactyla		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Felis catus		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Acinonyx jubatus		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Panthera uncia		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Panthera tigris		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Canis lupus		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Ursus maritimus		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Ailuropoda melanoleuca		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Mustela putorius		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Odobenus rosmarus		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Leptonychotes weddellii		M	G	I	S	T	R	E	L	F	L	N	F	T	I	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Tapirus indicus		M	E	W	T	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Ceratotherium simum		M	E	W	T	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Diceros bicornis		M	E	W	T	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	?	
Rhinoceros unicornis		M	E	W	T	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Dicerorhinus sumatrensis		M	E	W	T	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Coelodonta antiquitatis		M	E	W	T	T	R	E	L	F	L	N	F	T	V	V	L	I	T	V	I	L	M	W	L	L	V	R	S	Y	Q	Y	
Equus prezwalskii		M	E	W	R	-	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	-	
Equus caballus		M	E	W	R	-	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	-	
Equus asinus		M	E	W	R	-	R	E	L	F	L	N	F	T	V	V	L	I	T	V	L	L	M	W	L	L	V	R	S	Y	Q	-	
Camelus ferus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Vicugna pacos		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Sus scrofa		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Sus cebifrons		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Sus verrucosus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Balaenoptera acutorostrata		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Balaenoptera physalus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Balaenoptera bonaerensis		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Physeter macrocephalus 1 & 2		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Lipotes vexillifer		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Orcinus orca		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Tursiops truncates		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Capreolus capreolus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	

Pantholops hodgsonii		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Ovis aries		M	E	R	S	A	R	E	I	C	L	N	F	T	V	V	L	I	T	V	M	L	I	W	L	L	V	R	S	Y	Q	Y	
Capra hircus		M	E	R	S	T	R	E	I	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Capra aegagrus		M	E	R	S	T	R	E	I	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Bubalus bubalis		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Bison bison		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Bos grunniens		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Bos indicus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	
Bos taurus		M	E	R	S	T	R	E	L	C	L	N	F	T	V	V	L	I	T	V	I	L	I	W	L	L	V	R	S	Y	Q	Y	

fig. S4. Amino acid alignment of vertebrate sln. The mutated start codons (ATG→CTG) in the two-toed sloth (*Choleopus hoffmanii*) and brown-throated sloth (*Bradypus variegatus*) were verified from the NCBI sequence read archive and polymerase chain reaction amplification, respectively. Amino acid deletions in the equid lineage are highlighted in red. Note that two sarcolipin gene copies were found in the *Xenopus tropicalis*, *Monodelphis domestica*, and *Physeter macrocephalus* assemblies. Missing data denoted by question marks.

table S1. GenBank accession numbers of species used in this study.

Species name	***ucp1* Source**	***ucp2* Source**	***ucp3* Source**	***sln* Source**
Osteichthyes				
Astyanax mexicanus	APWO01100404.1 APWO01100403.1	APWO01075838.1	APWO01075838.1	Missing
Cyprinus carpio	AY461434.2	AJ243486.1	AY505343.1	Missing
Danio rerio	CABZ01073633.1 CABZ01073634.1	CABZ01017713.1	CABZ01012840.1 CABZ01017713.1	NM_001302745.1
Latimeria chalumnae	AFYH01226130.1 AFYH01226129.1	BAHO01089108.1 AFYH01155949.1	Missing	AFYH01102483.1
Lepisosteus oculatus	AHAT01015452.1	AHAT01003069.1	AHAT01003069.1	AHAT01003063.1
Takifugu rubripes	CAAB02001533.1	CAAB02000575.1	CAAB02000575.1	CAAB02007733.1
Xiphophorus maculatus	AGAJ01004818.1 AGAJ01004819.1	AGAJ01021216.1	AGAJ01021216.1	AGAJ01040366.1
Amphibia				
Xenopus tropicalis	AAMC02004107.1	NM_203848.1	NM_001095178.1	AAMC02008311.1 AAMC02008313.1
Montremata				
Ornithorhynchus anatinus	NW_001794248.1	AAPN01056149.1 AAPN01056148.1 AAPN01056147.1 AAPN01382618.1	AAPN01178604.1 AAPN01178605.1	AAPN01035927.1
Marsupialia				
Monodelphis domestica	AAFR03015618.1	AAFR03050488.1	AAFR03050488.1	AAFR03009954.1 AAFR03043596.1
Macropus eugenii	ABQO020217652.1 ABQO020162103.1 ABQO020106554.1 ABQO020051005.1 ABQO021106434.1	ABQO020850797.1 ABQO020012246.1	ABQO020339531.1 ABQO020395080.1 ABQO020450629.1 ABQO020707648.1 ABQO020652099.1	ABQO020365261.1
Sarcophilus harrisii	AEFK01228715.1 AEFK01215941.1	AFEY01422591.1	AFEY01409403.1 AFEY01344314.1 AFEY01422590.1	AFEY01139015.1
Sminthopsis crassicaudata	EF622232.1	Missing	Missing	Missing
Xenarthra				
Choloepus hoffmanni	ABVD02364175.1	ABVD01312484.1	ABVD02315747.1	ABVD02284044.1
Bradypus variegatus	KY886638	Missing	Missing	KY886670
Cyclopes didactylus	KY886639	Missing	Missing	Missing
Dasypus novemcinctus	AAGV03181320.1	AAGV03150769.1	AAGV03292997.1 AAGV03292996.1	AAGV03173212.1
Mylodon darwinii	SRX327588	SRX327588 SRX327589	SRX327588 SRX327589 SRX465052	SRX327588

Afrotheria

Chrysochloris asiatica	AMDV01244955.1 AMDV01244956.1	AMDV01302651.1	AMDV01302653.1	AMDV01027313.1
Dugong dugon	KY886640	KY886652	KY886661	KY886671
Echinops telfairi	AAIY02209271.1	AAIY02169770.1	AAIY02169772.1	AAIY02172361.1
Elephantulus edwardii	AMGZ01097263.1	AMGZ01085574.1	AMGZ01085576.1; AMGZ01085575.1	AMGZ01226582.1
Elephas maximus	KY886642	ERX334764 ERX334765	ERX334764 ERX334765	ERX334764 ERX334765
Hydrodamalis gigas	KY886641	KY886653	KY886662	KY886672
Loxodonta africana	AAGU03034821.1	AAGU03079300.1	AAGU03079300.1	AAGU03058675.1
Mammuthus primigenius	ERP008929	ERP008929	ERP008929	ERP008929
Orycteropus afer	ALYB01104541.1 ALYB01104543.1	ALYB01103367.1	ALYB01103365.1	ALYB01161748.1
Procavia capensis	ABRQ02143236.1	ABRQ02011699.1	ABRQ02011697.1	ABRQ02037372.1
Trichechus manatus latirostris	AHIN01109623.1 AHIN01109624.1	AHIN01084584.1	AHIN01084587.1	AHIN01088368.1

Laurasiatheria

Acinonyx jubatus	LLWD01000416.1	LLWD01000509.1	LLWD01000509.1	LLWD01000070.1
Ailuropoda melanoleuca	ACTA01016457.1	ACTA01146014.1	ACTA01130015.1	ACTA01090054.1
Balaena mysticetus	KY886643	Missing	Missing	Missing
Balaenoptera acutorostrata	ATDI01065547.1	ATDI01094623.1	ATDI01094627.1	ATDI01043778.1
Balaenoptera bonaerensis	BAUQ01197845.1 BAUQ01696173.1 BAUQ01341929.1	BAUQ01410165.1	BAUQ01284797.1	BAUQ01341977.1
Balaenoptera physalus	SRX323050	SRX323050	SRX323050	SRX323050
Bison bison	JPYT01100523.1	JPYT01642782.1	JPYT01019071.1	JPYT01254401.1
Bos grunniens	AGSK01075302.1	AGSK01058532.1 [Misassembled]	AGSK01132661.1 AGSK01132663.1 [Misassembled]	AGSK01136949.1
Bos indicus	AGFL01142555.1 AGFL01142556.1	AGFL01131821.1	AGFL01131836.1 AGFL01131835.1	AGFL01128732.1
Bos taurus	DAAA02044420.1 DAAA02044421.1	AAFC03092999.1	NM_174210.1	AAFC03004956.1
Bubalus bubalis	AWWX01630119.1	AWWX01596091.1	AWWX01532946.1	AWWX01539210.1
Camelus ferus	AGVR01051296.1	AGVR01023127.1	AGVR01023127.1	AGVR01041834.1
Canis lupus familiaris	NM_001003046.1	AAEX03012810.1	NM_001003047.1	AOCS01147984.1
Capra aegagrus	CBYH010071014	CBYH010041467.1	CBYH010041467.1	JXYW01145092.1
Capra hircus	AJPT01162993.1	JN986832.1	AJPT01151377.1	AJPT01148506.1
Capreolus capreolus	CCMK010104759.1 CCMK012865005.1 CCMK010278719.1	CCMK010138775.1	CCMK010008945.1	CCMK010366203.1
Ceratotherium simum	AKZM01017598.1	AKZM01032604.1	AKZM01032604.1	AKZM01019766.1
Choeropsis liberiensis	KY886644	KY886654	KY886663	Missing
Coelodonta antiquitatis	KY886645	KY886655	KY886664	KY886673
Condylura cristata	AJFV01047153.1	AJFV01042335.1	AJFV01042336.1	AJFV01057960.1
Delphinapterus leucas	KY886646	KY886656	KY886665	Missing

Diceros bicornis	KY886647	KY886657	KY886666	KY886674
Dicerorhinus sumatrensis	KY886648	KY886658	KY886667	KY886675
Eidolon helvum	AWHC01029981.1	AWHC01218500.1	AWHC01218502.1 AWHC01218501.1	AWHC01196379.1
Eptesicus fuscus	ALEH01005956.1	ALEH01084492.1	ALEH01084495.1; ALEH01084494.1	ALEH01120006.1
Equus asinus	SRX290673 SRX290675 SRX290677	SRX290673 SRX290675 SRX290677	SRX290673 SRX290675 SRX290677	ERX607030 ERX607036 ERX607001
Equus caballus	AAWR02018851.1	AAWR02018167.1	AAWR02018167.1	AAWR02020714.1
Equus przewalskii	ATBW01036321.1 ATBW01036320.1	ATBW01058700.1 ATBW01058701.1	ATBW01058699.1	ATBW01057363.1
Equus quagga boehmi	KY886649	Missing	Missing	Missing
Erinaceus europaeus	AMDU01193160.1 AMDU01193161.1 AMDU01193162.1	AANN01307683.1	AMDU01063251.1 AMDU01063252.1 AMDU01063253.1	AANN01527796.1
Felis catus	ACBE01132808.1 ACBE01132809.1	AANG02100994.1	AANG02100994.1	ACBE01332355.1
Leptonychotes weddellii	APMU01141180.1 APMU01115166.1	APMU01037391.1	APMU01037393.1	APMU01115847.1
Lipotes vexillifer	AUPI01000024.1 AUPI01000025.1	AUPI01017650.1	AUPI01017651.1	AUPI01060449.1
Manis pentadactyla	JPTV01131901.1	JPTV01099221.1	JPTV01146288.1	JPTV01082441.1
Megaderma lyra	AWHB01348443.1 AWHB01348444.1	AWHB01174334.1 AWHB01174333.1	AWHB01278871.1 AWHB01278872.1	AWHB01215178.1
Mustela putorius furo	AGTQ01041845.1	AGTQ01009941.1	AGTQ01009939.1	AEYP01061363.1
Myotis brandtii	ANKR01273868.1 ANKR01273869.1	ANKR01210024.1	ANKR01210023.1	ANKR01318413.1
Myotis davidii	ALWT01125743.1	ALWT01314732.1 ALWT01314733.1	ALWT01314729.1	ALWT01118440.1
Myotis lucifugus	AAPE02001462.1	AAPE02040816.1	AAPE02040815.1 AAPE02040816.1	AAPE02047638.1
Neovison vison	EU360729.1	EU360730.1	Missing	Missing
Odobenus rosmarus	ANOP01028105.1	ANOP01000820.1	ANOP01000821.1	ANOP01017035.1
Orcinus orca	ANOL02004931.1 (Deleted gene - Synteny analysis)	ANOL02054064.1	ANOL02054065.1	ANOL02064465.1
Ovis aries	JN604985.1	CBYI010055183.1	CBYI010055184.1	ACIV011354444.1
Panthera tigris altaica	ATCQ01112915.1	ATCQ01048793.1	ATCQ01048791.1	ATCQ01029167.1
Panthera uncia	SRX273036	SRX273036	SRX273036	SRX273036
Pantholops hodgsonii	AGTT01188813.1	AGTT01169141.1	AGTT01169136.1	AGTT01037849.1
Phoca vitulina	AB921529.1	Missing	Missing	Missing
Physeter macrocephalus	AWZP01062081.1	AWZP01062236.1	AWZP01062236.1	AWZP01043685.1 AWZP01078901.1
Pteropus alecto	ALWS01011689.1	ALWS01169197.1	ALWS01169198.1 ALWS01169197.1	ALWS01050977.1
Pteropus vampyrus	ABRP02126915.1	ABRP02138358.1	ABRP02138358.1	ABRP02100214.1
Rhinoceros unicornis	KY886650	KY886659	KY886668	KY886676
Rhinolophus	AWHA01040305.1	AWHA01096410.1	AWHA01092973.1	AWHA01130749.1

ferrumequinum	AWHA01040304.1		AWHA01092975.1	
Sorex araneus	AALT02056093.1	AALT02155451.1	AALT02155448.1 AALT02155449.1	AALT02042376.1
Suncus murinus	AB244816.1	Missing	Missing	Missing
Sus cebrifrons	ERX149172	ERX149172	ERX149172	ERX149172
Sus scrofa	DQ372918.1	NM_214289.1	NM_214049.1	AJKK01191606.1
Sus verrucosus	ERX1054048-ERX1054067	ERX1054048-ERX1054067	ERX1054048-ERX1054067	ERX1054048-ERX1054067
Tapirus indicus	KY886651	KY886660	KY886669	KY886677
Tursiops truncatus	ABRN02199412.1 (Deleted gene - Synteny analysis)	ABRN02011700.1	ABRN02011697.1	ABRN02379235.1
Ursus maritimus	AVOR01014285.1	AVOR01090225.1	AVOR01090228.1	AVOR01032007.1
Vicugna pacos	ABRR02134987.1	ABRR02047266.1	ABRR02047266.1	ABRR02146164.1

Euarchontoglires

Aotus nancymaae	JYKP01215429.1	JYKP01068224.1	JYKP01068227.1	JYKP01155709.1
Apodemus sylvaticus	LIPJ01014497.1 LIPJ01184746.1	LIPJ01041350.1	LIPJ01000433.1	LIPJ01004285.1
Callithrix jacchus	ACFV01002817.1 ACFV01002818.1	ACFV01035072.1	ACFV01035065.1 ACFV01035066.1 ACFV01035067.1	JRUL010233331.1
Cavia aperea	AVPZ01000778.1	AVPZ01000005.1	AVPZ01000005.1	AVPZ01000041.1
Cavia porcellus	AAKN02011801.1	AAKN02051191.1	AAKN02051190.1	AAKN02053822.1
Cercocebus atys	JZLG01060688.1	JZLG01056565.1	JZLG01056561.1	JZLG01010897.1
Chinchilla lanigera	AGCD01027651.1 AGCD01027652.1	AGCD01015245.1	AGCD01015243.1	AGCD01012756.1
Chlorocebus sabaeus	AQIB01017419.1	AQIB01134135.1	AQIB01134133.1	AQIB01093034.1
Colobus angolensis	JYKR01122839.1 JYKR01122838.1 JYKR01122837.1	JYKR01062401.1	JYKR01062397.1	JYKR01171003.1
Cricetulus griseus	AFTD01128394.1	AFTD01106142.1	AFTD01106142.1	APMK01041211.1
Daubentonia madagascariensis	AGTM011584996.1 AGTM011708528.1 AGTM012010142.1 AGTM011594144.1	AGTM010366851.1 AGTM012942927.1 AGTM010409851.1 AGTM011996042.1 AGTM011949823.1	AGTM010372645.1 AGTM011680642.1	AGTM011625695.1
Dicrostonyx groenlandicus	AF515781.1	Missing	Missing	Missing
Dipodomys ordii	ABRO02057411.1	ABRO02020382.1	ABRO02020382.1	ABRO02010842.1
Eulemur flavifrons	LGHW01000184.1	LGHW01005764.1	LGHW01005764.1	LGHW01001219.1
Eulemur macaco	LGHX01000184.1	LGHX01005675.1	LGHX01005675.1	LGHX01001209.1
Fukomys damarensis	AYUG01151055 AYUG01151056	AYUG01134562.1 AYUG01134563.1	AYUG01134566.1 AYUG01134565.1	AYUG01150094.1
Galeopterus variegatus	JMZW01045216.1 JMZW01045217.1	JMZW01159785.1 JMZW01159786.1	JMZW01159781.1	JMZW01093471.1
Gorilla gorilla gorilla	NW_004002547.1	NW_004005093.1	NW_004005093.1	CABD030081410.1
Heterocephalus glaber	AFSB01162372.1	AFSB01032172.1	AFSB01032171.1	AFSB01080593.1
Homo sapiens	NG_012139.1	U82819.1	U84763.1	U96094.1

Jaculus jaculus	AKZC01091543.1	AKZC01100636.1 AKZC01100637.1	AKZC01100628.1 AKZC01100629.1 AKZC01100630.1 AKZC01100632.1	AKZC01057088.1
Macaca fascicularis	CAEC01514737.1	AEHL01150243.1	AEHL01150254.1; AEHL01150253.1	CAEC01142624.1
Macaca mulatta	AANU01271750.1	AANU01189618.1	AANU01189616.1	AEHK01077455.1
Macaca nemestrina	JZLF01028562.1	JZLF01057018.1	JZLF01057022.1	JZLF01029339.1
Mandrillus leucophaeus	JYKQ01107155.1 JYKQ01107156.1	JYKQ01166891.1	JYKQ01166885.1	JYKQ01102438.1
Marmota marmota	CZRN01000015.1	CZRN01000004.1	CZRN01000004.1	CZRN01000026.1
Mesocricetus auratus	NM_001281332.1	APMT01084061.1	APMT01084061.1	APMT01047702.1
Microcebus murinus	ABDC01082367.1	ABDC01179060.1	ABDC01179052.1 ABDC01179053.1	ABDC02015405.1
Microtus agrestis	LIQJ01004042.1	LIQJ01014426.1	LIQJ01014426.1	LIQJ01000448.1
Microtus ochrogaster	AHZW01157106.1	AHZW01107615.1	AHZW01107615.1	AHZW01020748.1
Mus musculus	CAAA01024310.1	CAAA01006333.1	AAHY01064811.1	AAHY01078220.1
Myodes glareolus	LIPI01003929.1	LIPI01011290.1	LIPI01011290.1	LIPI01022398.1
Nannospalax galili	AXCS01128925.1	AXCS01188952.1	AXCS01188951.1	AXCS01045434.1
Nasalis larvatus	JMHX01319533.1	JMHX01319543.1	CM003003.1	JMHX01319543.1
Nomascus leucogenys	ADFV01177960.1 ADFV01177959.1	ADFV01111583.1	ADFV01111578.1 ADFV01111580.1	ADFV01056290.1
Ochotona dauurica	AB283043.1	Missing	Missing	Missing
Ochotona princeps	ALIT01060999.1	AAYZ01137505.1	AAYZ01237828.1	AAYZ01218708.1
Octodon degus	AJSA01193670.1 AJSA01193671.1	AJSA01174660.1	AJSA01174659.1 AJSA01174660.1	AJSA01031476.1
Oryctolagus cuniculus	NM_001171077.1	AAGW02008192.1	AAGW02008192.1	AAGW02009195.1
Otolemur garnettii	AAQR03074138.1	AAQR03001165.1	AAQR03001162.1	AAQR03181843.1
Pan paniscus	AJFE01070904.1		AJFE01047917.1	AJFE01100549.1
Pan troglodytes	AACZ03032212.1 AACZ03032211.1	AACZ03079660.1	AADA01222375.1	AADA01279835.1
Papio anubis	AHZZ01043343.1	AHZZ01024419.1	AHZZ01024424.1	AHZZ01040054.1
Papio hamadryas	PreEnsembl	Missing	Missing	Missing
Peromyscus maniculatus	AYHN01134223.1 AYHN01134224.1	AYHN01057054.1	AYHN01057054.1	AYHN01010930.1
Phodopus sungorus	AF271263.1	AF271264.1	AF271265.2	Missing
Pongo abelii	ABGA01062109.1 ABGA01062111.1	ABGA01223176.1 ABGA01223175.1	ABGA01223178.1	ABGA01383648.1
Propithecus coquereli	JZKE01017273.1 JZKE01017272.1 JZKE01017271.1	JZKE01114303.1	JZKE01114303.1	JZKE01236593.1
Rattus norvegicus	AAHX01097782.1	AAHX01007471.1	AABR06007535.1	AAHX01054738.1
Rhinopithecus roxellana	JABR01098768.1	JABR01036514.1	JABR01036511.1	JABR01016757.1
Saimiri boliviensis	AGCE01051213.1	AGCE01022106.1	AGCE01022109.1	AGCE01023439.1
Spermophilus tridecemlineatus	AGTP01049379.1	AAQQ01149310.1	AGTP01028875.1	AAQQ01672914.1
Tarsius syrichta	ABRT02355485.1 ABRT02355484.1	ABRT02455082.1	ABRT02455086.1 ABRT02455085.1	ABRT02333009.1
Tupaia belangeri chinensis	ALAR01031044.1	ALAR01010153.1	ALAR01010154.1	ALAR01067265.1

table S2. Likelihood ratio tests for *ucp1* CODEML models. The M2 pseudogene branch model was tested against individual branch models (M2), with additional branch categories for the camel, pygmy hippopotamus, stem rhinoceros, and stem pinniped branches. The pseudogene branch model (M2) analyses with and without the stem placental branch as its own category were tested against each other showing that ω of the stem placental branch (ω = 0.6333) is significantly different than all placental branches with functional *ucp1* (ω = 0.1591). Branch categories for the pseudogene analysis included non-mammalian vertebrates, non-placental mammals, placental branches with intact *ucp1* loci, individual transitional branches (M2 ω values displayed in Fig. 3), and pseudogenic branches. Site and branch-site models were performed with pseudogenes pruned from the dataset. The site model M2a revealed no sites under positive selection and no difference from the null model M1a. The branch site model A was performed to identify sites on the stem placental branch that may have contributed to the gain of NST function. While eight sites were found to have a posterior probability >0.95, model A was not significantly different from the corresponding null model where ω is fixed at 1 for foreground branch sites in the positive selection site category. Site and branch-site model parameters include the proportion of sites (p_0, p_1, p_2, p_{2a}, and p_{2a}) and omega values (ω_0, ω_1, ω_2, ω_{2a} and ω_{2b}) for all site classes.

LRT models	Parameter estimates	2Δℓ	df	p-value	Sites (posterior probability >0.95)
Branch models					
M2 pseudogene vs. M2 camel branch	**M2 pseudogene:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1591$ $\omega_{\text{pseudogenic placental branches}} = 0.9443$ **M2 camel branch:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1585$ $\omega_{\text{pseudogenic placental branches}} = 0.9438$ $\omega_{\textit{C. ferus}} = 1.0330$	4.666142	1	0.030762959	
M2 pseudogene vs. M2 pygmy hippo branch	**M2 pseudogene:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1591$ $\omega_{\text{pseudogenic placental branches}} = 0.9443$ **M2 pygmy hippo branch:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1585$ $\omega_{\text{pseudogenic placental branches}} = 0.9439$ $\omega_{\textit{C. liberiensis}} = 0.2530$	1.454692	1	0.227776571	

M2 pseudogene vs. M2 stem pinniped branch	**M2 pseudogene:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1591$ $\omega_{\text{pseudogenic placental branches}} = 0.9443$ **M2 pinniped branch:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1580$ $\omega_{\text{pseudogenic placental branches}} = 0.9439$ $\omega_{\text{stem pinniped branch}} = 0.2866$	0.074304175	1	0.074304175	
M2 pseudogene vs. M2 stem rhinoceros branch	**M2 pseudogene:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1591$ $\omega_{\text{pseudogenic placental branches}} = 0.9443$ **M2 stem rhinoceros branch:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1585$ $\omega_{\text{pseudogenic placental branches}} = 0.9438$ $\omega_{\text{stem rhinoceros branch}} = 0.2851$	1.459344	1	0.227034526	
M2 pseudogene vs. M2 stem placental branch	**M2 pseudogene:** $\omega_{\text{functional non-mammal branches}} = 0.0501$ $\omega_{\text{functional non-placental branches}} = 0.1238$ $\omega_{\text{functional placental branches}} = 0.1591$ $\omega_{\text{pseudogenic placental branches}} = 0.9443$ **M2 stem placental branch:** $\omega_{\text{functional non-mammal branches}} = 0.0502$ $\omega_{\text{functional non-placental branches}} = 0.1183$ $\omega_{\text{functional placental branches}} = 0.1536$ $\omega_{\text{pseudogenic placental branches}} = 0.9437$ $\omega_{\text{stem placental branch}} = 0.6333$	36.676754	1	1.39432E-9	
Site models					
M1a vs. M2a	**M1a:** $p_0 = 0.92037$ $p_1 = 0.07963$ $\omega_0 = 0.11041$ $\omega_1 = 1.00000$ **M2a:** $p_0 = 0.92037$ $p_1 = 0.05616$ $p_2 = 0.02347$ $\omega_0 = 0.11038$ $\omega_1 = 1.00000$ $\omega_2 = 1.00004$	0	2	1	None
Branch-site models					
Model A null vs. Model A - stem placental branch	**Model A null:** $p_0 = 0.47004$ $p_1 = 0.04251$ $p_{2a} = 0.44702$ $p_{2b} = 0.04043$ $\omega_0 \ \& \ \omega_{2a} \text{ background} = 0.10531$ $\omega_1 \ \& \ \omega_{2b} \text{ background} = 1.00000$ $\omega_0 \text{ foreground} = 0.10531$ $\omega_1, \ \omega_{2a} \ \& \ \omega_{2b} \text{ foreground} = 1.00000$ **Model A:** $p_0 = 0.54398$ $p_1 = 0.04910$ $p_{2a} = 0.37323$ $p_{2b} = 0.03369$ $\omega_0 \ \& \ \omega_{2a} \text{ background} = 0.10532$	0.63447	1	0.425720414	15L, 22S, 76K, 124L, 153I, 170T, 224I, 261M

ω_{1} & ω_{2b} background = 1.00000
ω_{0} foreground = 0.10532
ω_{1} foreground = 1.00000
ω_{2a} & ω_{2b} foreground = 1.40522

table S3. Calculated *ucp1* inactivation dates (Ma) within the placental mammal lineage. Inactivation date ranges were calculated using the two sets of formulas specified by Meredith *et al*. (*26*) with branch model CODEML analyses using species divergences and branch lengths arising from our 51 kb maximum likelihood species timetree analysis (data file S1). The first dating method assumes the rate of "synonymous substitution are neutral and equal on functional and pseudogene branches" while the second assumes "the rate synonymous substitution on functional branches is non-neutral and 70% of the rate of synonymous substitution on pseudogene branches". Inactivation date calculations utilized ω values for placental branches with functional *ucp1* (ω=0.1591), transitional branches (ω values displayed in Fig. 3), and pseudogenic branches (ω=0.9435). As ω values calculated for *Dasypus novemcinctus*, *Manis pentadactyla*, and the stem cetacean branch (ω=1.0567, 0.9849, and 0.9872, respectively) exceeded the mean value of pseudogenized branches, we placed their inactivation dates at the base of each clade. Conversely, the ω value (0.0805) for the stem Pilosa branch was below that for functional branches; shared inactivating mutations in three-toed sloth (*Bradypus*), two-toed sloth (*Choleopus*), and silky anteater (*Cyclopes didactylus*), however, indicate *ucp1* was pseudogenized prior to their radiation ~55.03 Ma. Note that since only remnants of *ucp1* (exon 6) were identified from representative Pilosa, it is possible that *ucp1* pseudogenization preceded its divergence from Cingulata.

Transitional branch	**Inactivation date (Ma)**
Dasypus novemcinctus	65.55
Pilosa	>55.03
Procavia capensis	33.5–27.3
Proboscidea	42.9–37.5
Sirenia	46.0–44.8
Suidae (*Sus spp.*)	41.2–36.4
Cetacea	52.75
Equus	25.1–20.7
Manis pentadactyla	74.69

table S4. Results of BiSSE models with and without constraints on the diversification rate for five trees from the work of Faurby and Svenning (*56*).

Tree #	Model	Constraints	lambda0	lambda1	mu0	mu1	q01	q10	parameters	Ln Lik	AIC	p value
1	No reversal	q10 = 0	0.2990537	0.2118425	0.2301557	0.1818380	0.0001916	0	5	-14264	28539	2.32E-05
1	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.2985928	0.2985928	0.2333964	0.2333964	0.0001907	0	3	-14275	28556	
2	No reversal	q10 = 0	0.2979023	0.1557338	0.2310627	0.1124779	0.0001479	0	5	-14334	28679	1.31E-07
2	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.292754	00.292754	0.228024	0.228024	0.0001891	0	3	-14350	28707	
3	No reversal	q10 = 0	0.2724713	0.1950414	0.1966417	0.1611570	0.0001922	0	5	-14467	28944	1.27E-05
3	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.2714773	0.2714773	0.1993568	0.1993568	0.0001890	0	3	-14478	28963	
4	No reversal	q10 = 0	0.3102003	0.1427713	0.2429003	0.0985164	0.0001410	0	5	-14178	28366	9.45E-10
4	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.3041617	0.3041617	0.2393333	0.2393333	0.0001951	0	3	-14199	28404	
5	No reversal	q10 = 0	0.2789428	0.1652951	0.2083545	0.1242601	0.0001596	0	5	-14518	29047	3.37E-06
5	No reversal, equal diversification	lambda0 = lambda1, mu0 = mu1, q10 = 0	0.275516y	0.2755167	0.2074114	0.2074114	0.0001844	0	3	-14531	29068	

table S5. BiSSE results for neutral character simulations. Neutral characters were simulated on the same five trees (5138 taxa) as in table S4. In each case, a model with no reversal (q10 = 0) and no constraints on diversification rates was compared to a model with no reversal (q10 = 0), equal speciation rates (lambda0 = lambda 1), and equal extinction rates (mu0 = mu1). Significance levels are indicated (* = p<0.5; ** = p<0.01; *** = p<0.0001).

Tree #	Ln Lik	AIC	ChiSq	p value	Significance level
1	-14287	28580	8.3649	0.01526	*
1	-14290	28585	17.882	0.0001309	***
1	-14283	28571	25.832	2.46E-06	***
1	-14268	28541	1.6061	0.448	
1	-14297	28600	5.7015	0.0578	
1	-14279	28564	2.7851	0.2484	
1	-14268	28543	6.1693	0.04575	*
1	-14279	28564	0.68013	0.7117	
1	-14267	28539	1.6509	0.438	
1	-14288	28582	2.1826	0.3358	
1	-14262	28531	1.4547	0.4832	
1	-14311	28628	0.24268	0.8857	
1	-14268	28543	1.2025	0.5481	
1	-14291	28588	15.569	0.0004162	***
1	-14254	28514	4.3205	0.1153	
1	-14268	28542	8.56	0.01384	*
1	-14284	28574	5.711	0.05753	
1	-14304	28615	4.7037	0.0952	
1	-14276	28558	15.226	0.0004939	***
1	-14301	28608	2.1298	0.3448	
2	-14321	28648	2.2439	0.3256	
2	-14352	28709	12.725	0.001725	**
2	-14368	28742	1.9207	0.3828	
2	-14360	28725	0.30942	0.8567	
2	-14360	28726	2.7076	0.2583	
2	-14342	28690	7.9521	0.01876	*
2	-14332	28669	3.5106	0.1729	
2	-14321	28648	2.2393	0.3264	
2	-14356	28717	2.5266	0.2827	
2	-14340	28686	1.5311	0.4651	
2	-14340	28687	3.4521	0.178	
2	-14347	28700	11.566	0.00308	**
2	-14339	28685	7.5964	0.02241	*
2	-14355	28715	12.958	0.001535	**
2	-14348	28703	13.494	0.001174	**
2	-14354	28714	3.9504	0.1387	

2	-14349	28704	19.476	5.90E-05	***
2	-14360	28727	2.1532	0.3408	
2	-14348	28703	15.847	0.0003622	***
2	-14353	28713	0.42811	0.8073	
3	-14447	28899	24.473	4.85E-06	***
3	-14509	29024	4.2386	0.1201	
3	-14525	29056	16.217	0.0003009	***
3	-14488	28982	7.0465	0.0295	*
3	-14514	29034	7.2817	0.02623	*
3	-14494	28995	0.22603	0.8931	
3	-14470	28947	1.5699	0.4561	
3	-14498	29001	3.105	0.2117	
3	-14481	28967	7.1922	0.02743	*
3	-14472	28950	3.9134	0.1413	
3	-14477	28961	2.3794	0.3043	
3	-14472	28950	0.68295	0.7107	
3	-14455	28916	6.5718	0.03741	*
3	-14500	29007	17.326	0.0001729	***
3	-14477	28960	14.934	0.0005715	***
3	-14471	28947	13.084	0.001441	**
3	-14504	29014	3.9079	0.1417	
3	-14523	29051	7.2379	0.02681	*
3	-14476	28958	23.591	7.54E-06	***
3	-14501	29008	1.8208	0.4024	
4	-14178	28362	0.90767	0.6352	
4	-14220	28446	25.795	2.51E-06	***
4	-14212	28431	7.0962	0.02878	*
4	-14214	28433	18.623	9.04E-05	***
4	-14208	28423	0.68958	0.7084	
4	-14210	28426	22.513	1.29E-05	***
4	-14188	28382	1.5229	0.467	
4	-14215	28436	6.0754	0.04794	*
4	-14202	28410	4.4862	0.1061	
4	-14187	28380	1.037	0.5954	
4	-14200	28406	26.321	1.93E-06	***
4	-14187	28380	2.1832	0.3357	
4	-14176	28358	2.4273	0.2971	
4	-14187	28380	6.1279	0.0467	*
4	-14191	28388	8.889	0.01174	*
4	-14199	28403	9.5794	0.008315	**
4	-14188	28382	13.933	0.0009428	***
4	-14213	28432	20.863	2.95E-05	***
4	-14194	28393	4.8134	0.09011	

4	-14200	28405	5.7027	0.05777	
5	-14527	29060	1.4141	0.4931	
5	-14546	29098	3.5835	0.1667	
5	-14536	29078	5.8842	0.05275	
5	-14549	29105	2.5866	0.2744	
5	-14525	29057	1.59	0.4516	
5	-14555	29116	107.64	<2.2e-16	***
5	-14550	29106	0.29186	0.8642	
5	-14526	29058	4.8953	0.0865	
5	-14557	29119	2.5245	0.283	
5	-14530	29066	1.7002	0.4274	
5	-14546	29098	8.3536	0.01535	*
5	-14552	29109	4.0583	0.1314	
5	-14545	29096	4.2978	0.1166	
5	-14546	29098	3.0219	0.2207	
5	-14534	29074	10.38	0.005572	**
5	-14510	29025	9.9686	0.006845	**
5	-14570	29147	32.984	6.88E-08	***
5	-14562	29129	6.778	0.03374	*
5	-14545	29096	4.8771	0.08729	
5	-14526	29058	0.23807	0.8878	

table S6. Deleterious mutations found in *ucp2* and *ucp3* sequences of placental mammal taxa. Base numbers correspond to position in multiple species alignments (data files S7 and S8). Note that the coding region of several *ucp2* and *ucp3* sequences in GenBank (*ucp2*: Tammar wallaby [ABQO020850797.1], orangutan [ABGA01223176.1, ABGA01223175.1], and thirteen-lined ground squirrel [AAQQ01149310.1]; *ucp3*: Northern white-cheeked gibbon [ADFV01111578.1]) showed signs of disruption (indels), though these were not present in the NCBI sequence read archive and thus likely represent assembly errors.

Gene	Species name	Base number	Inactivating mutation
ucp2			
	Dasypus novemcinctus	1	Mutated initiation codon
		96	1 bp deletion
		348-349	2 bp deletion
		396-397	2 bp deletion
		463	1 bp deletion
		646-648	Nonsense mutation
		653-654	2 bp deletion
		673	1 bp deletion
		889	1 bp deletion
		906	1 bp deletion
		930	1 bp deletion
ucp3			
	Dasypus novemcinctus	752	1 bp deletion
	Oryctolagus cuniculus	54	1 bp deletion
		78	1 bp deletion

table S7. Specimen data and sources of tissue samples used for PCR amplification and DNA hybridization capture.

Common name	Scientific name	Sample	Provided by
Xenarthra			
Brown-throated sloth	*Bradypus variegatus*	Genomic DNA, previously extracted	M. Springer, University of California, Riverside
Silky anteater	*Cyclopes didactylus*	Genomic DNA, previously extracted (*46*)	M. Springer, University of California, Riverside
Paenungulata			
Dugong	*Dugong dugon*	Two genomic DNA samples, Female, Mabuiag Island, Torres Straits, Australia, previously extracted (*69*); Sample codes: MD33 and MD118	D. McDonald, James Cook University, Australia
Steller's sea cow	*Hydrodamalis gigas*	Three bone samples housed in the Zoological Institute of the Russian Academy of Sciences (St. Petersberg, Russian Federation), previously extracted (*64, 70*) ZI 6852: Mandible collected from Bering Island, Komandorskii in 1884 ZI6853: Humerus, no collection information ZI 17170: Scapula, no collection information	R. MacPhee, American Museum of Natural History
Asian elephant	*Elephas maximus*	Genomic DNA, Male, previously extracted (*69*)	W. Korver, Bowmanville Zoo, Ontario, Canada
African elephant	*Loxodonta africana*	Genomic DNA, Female, previously extracted (*69*)	W. Korver, Bowmanville Zoo, Ontario, Canada
Laurasiatheria			
Pygmy hippopotamus	*Choeropsis liberiensis*	Genomic DNA, previously extracted (*79*)	New York Zoological Society
Bowhead whale	*Balaena mysticetus*	Genomic DNA	SWFSC
Beluga whale	*Delphinapterus leucas*	Genomic DNA, previously extracted (*79*)	New York Zoological Society
Grant's zebra	*Equus quagga boehmi*	Skeletal muscle from stillborn calf	S. Petersen, Assiniboine Park Zoo, Manitoba, Canada
Malayan tapir	*Tapirus indicus*	Blood	M. Berteslen, Copenhagen Zoo, Frederiksberg, Denmark T. Gilbert and E. Willerslev, University of Copenhagen
Woolly rhinoceros	*Coelodonta antiquitatis*	Permafrost preserved tooth and bone samples housed at the Centre for GeoGenetics at the Natural History museum of Denmark (Copehagen, Denmark) CGG_1_000024: Tooth, Bol'shoi Lyakhovsky Island, Novaya Sibir' Island, Siberia, Russia CGG_1_000029: Bone, Badyarikha, Indigirka Lowland, Russia CGG_1_000031: Bone, Khetachan Creek Mine, Kolyma Lowland, Russia	T. Gilbert and E. Willerslev, University of Copenhagen
Black rhinoceros	*Diceros bicornis*	Genomic DNA, previously extracted	CRES
Sumatran rhinoceros	*Dicerorhinus sumatrensis*	Blood excised from a leech that fed on a Sumatran rhinoceros	R. Havmøller, T. Gilbert, and E. Willerslev, University of Copenhagen Leibniz-Institute for Zoo and Wildlife Research, Berlin, Germany
Indian rhinoceros	*Rhinoceros unicornis*	Genomic DNA, previously extracted	CRES

table S8. Fossil constraints used in timetree analysis.[A]

Constrained Clade	Min Age	Max Age	Basis for Max Age	Oldest Fossil	Comments/References
1. Metatheria	65.18	83.8	SB	*Peradectes* (Puercan); *Minoperadectes* (Early Wasatchian)	Horovitz *et al.* (*81*) reported Puercan *Peradectes* (age = 65.18 Ma) as the oldest crown metatherian and recovered this taxon as a stem didelphimorphian. Benton *et al.* (*78*) cited Horovitz *et al.* (*81*) in support of a younger age for Metatheria but failed to describe their rationale for excluding *Peradectes* from crown Metatheria.
2. Xenarthra	47.8	66.0	PB	*Riostegotherium*	*Riostegotherium* is from the Itaboraian (*82*). The Itaboraian was originally considered equivalent to the Selandian stage (*83*), but Woodburne *et al.* (*84*) suggested an early Eocene age (minimum = 47.8 Ma). The first outgroup to Xenarthra is Afrotheria, Boreoeutheria, or Epitheria, all of which have early Paleocene representatives.
3. Proboscidea (=*Loxodonta* to *Elephas*)	6.8	11.62	SB	*Loxodonta*	Vignaud *et al.* (*85*) reported *Loxodonta* in their faunal list for a hominid locality in Chad. Vignaud *et al.*'s (*85*) locality was radiometrically dated by Lebatard *et al.* (*86*) to have an age between 6.8 and 7.2 Ma. The oldest *Elephas* lineage fossils have a minimum age of 5.332 Ma (*87, 88*).
4. *Dugong* to *Hydrodamalis*	28.1	38.0	SB	*Crenatosiren*	The minimum age is based on the inclusion of early Oligocene taxa (*e.g., Crenatosiren olseni*) in crown Dugongidae (*64, 89*).
5. Sirenia (*Dugong* to *Trichechus*)	41.3	59.2	SB	*Eotheroides*	The minimum age is based on the inclusion of Lutetian age *Eotheroides aegyptiacum* in crown Sirenia (*64, 89-91*).
6. Tethytheria	59.2	72.3	SB	*Eritherium*	*Eritherium* is a stem proboscidean and is no younger than the late Selandian (*92*), which has a minimum age of 59.1 Ma.
7. Paenungulata	59.2	72.3	SB	*Eritherium*	*Eritherium* is a stem proboscidean and is no younger than the late Selandian (*92*), which has a minimum age of 59.1 Ma.
8. Chiroptera	47.8	66.0		*Dizzya*	*Dizzya* (Philistidae, Vespertilionoidea) is among the oldest crown chiropterans and is from the Ypresian (*93*). We used the top of the Ypresian as a minimum age for Yangochiroptera. Phillips (*94*) suggested a maximum age of 58.9 Ma for Chiroptera. The oldest bats (earliest Eocene, ~55 Ma) were already highly specialized fliers with fully developed wings (*95, 96*). Bats are one of the least common groups of mammals in the fossil record (*93, 95, 97, 98*), and in our view it is unnecessarily restrictive to enforce a maximum of 58.9 Ma on crown Chiroptera. Furthermore, molecular studies have rearranged the evolutionary tree for bats and recovered numerous higher-level clades that were previously unrecognized based on morphological data (*97, 99, 100*). The relationships of several early Eocene fossils (*e.g., Palaeochiropteryx, Archaeonycteris, Hassianycteris*), in turn, lack robust support, and their inclusion or exclusion from crown Chiroptera is sensitive to the enforcement of molecular scaffolds (*96, 97, 101*). Phylogenetic uncertainties, convergent evolution, and a depauperate fossil record conspire together to limit our understanding of the early evolutionary history of bats and caution against employing a maximum that is younger than the KPg boundary (66 Ma).
9. Megadermatidae to Rhinolophidae+ Hipposideridae	38.0	56	SB	*Hipposideros*	*Hipposideros* and *Rhinolophus* are both known from the Eocene of Europe (*102*). Eiting and Gunnell (*93*) indicate that *Hipposideros* first occurs in the Bartonian whereas *Rhinolophus* first occurs in the Priabonian.
10. Caniformia (=Canidae to Arctoidea)	38.0	56	SB	*Hesperocyon gregarious*	*Hesperocyon gregarius* fossils are known from the Duchesnean (~Bartonian) land mammal age in the middle Eocene (*103*). *H. gregarius* is a stem canid (*104*).
11. Ursidae to other Arctoidea	28.1	41.3	SB	*Cephalogale*	*Cephalogale* belongs to the ursid subfamily Hemicyoninae and has emerged as a stem ursid in phylogenetic analyses (*105*); also see

					Wang *et al.* (*106*). *Cephalogale* is known from the early Oligocene (*107*). We used the top of the early Oligocene as the minimum for Urside to other arctoids.
12. Musteloidea to Pinnipedea	23.03	38.0	SB/PB/PU	*Promartes, Enaliarctos*	*Mustelavus priscus* is first known from the latest Eocene (*108*). *M. priscus* is either a stem musteloid (*106, 108, 109*), and the oldest representative of the crown taxon that includes musteloids and pinnipeds, or a deeper lineage within Caniformes (*110*). Tomiya's analysis is at odds with molecular studies and recovered *Ursus* inside of crown Musteloidea. Nevertheless, given the uncertainty regarding *Mustelavus* based on Tomiya (*110*), we implemented a minimum of 23.03 Ma for the musteloid-pinniped split based on the oldest musteloid (*Promartes*) (*105, 108*) and oldest pinniped (*Enaliarctos*) (*111*), both of which are from the late Oligocene.
13. Pinnipedia	20.43	33.9	SB	*Desmatophoca brachycephala*	*Desmatophoca brachycephala* is Aquitanian (20.43-23.03 Ma) in age (*112*). The oldest stem pinnipeds are Chattian (*e.g.*, *E. tedfordi*). SB yields an older maximum than PB.
14. Carnivora	38.0	66.0	PU	*Hesperocyon gregarious*	*Hesperocyon gregarius* is a basal canid in constrained (*113*) and unconstrained (*104, 113*) analyses. Viverravids from the early Paleocene are probably stem carnivorans (*113*), but phylogenetic uncertainty allows for the possibility that they are stem feliforms (*114-116*). Benton *et al.* (*78*) also suggested a maximum of 66 Ma for Carnivora.
15. Perissodactyla	55.5	61.6	SB	*Hyracotherium*	*Hyracotherium* first appears in Wa-0 at the beginning of the Eocene (*117*) and is the oldest crown Perissodactyla.
16. Erinaceidae to Soricidae	61.6	83.8	SB	*Adunator*	*Adunator* is the oldest erinaceomorph (*118*) and is known from the Torrejonian (contained within Danian). The minimum age is 61.6 Ma and the maximum is the base of the Campanian (83.6 +/-0.2 = 83.8 Ma) based on SB.
17. Cetartiodactyla	52.5	66.0	PB/PU	*Himalayacetus*	*Himalayacetus* is from shallow benthic zone SB8, which has a minimum age of 52.5 Ma within the Ypresian (*53, 118*). Mesonychids are a possible sister taxon to Cetartiodactyla, or may even be nested with Cetartiodactyla as the sister to Cetacea (*119*). The oldest mesonychids are early Paleocene in age and establish a maximum age for Cetartiodactyla.
18. Whippomorpha	52.5	61.6	SB	*Himalayacetus*	*Himalayacetus* is from shallow benthic zone SB8, which has a minimum age of 52.5 Ma within the Ypresian (*53, 118*).
19. Cetacea	34.0	47.8	SB	*Llanocetus denticrenatus*	*Llanocetus* is known from the La Meseta Formation of Seymour Island, Antarctica, and is latest Eocene in age (*53, 120*). *Llanocetus* is the oldest crown cetacean and a primitive stem mysticete. The oldest odontocetes are stem odontocetes from the Rupelian (early Oligocene) or more precisely from the late early Oligocene (*53*), although there are also older forms that have not been described. Uhen (*53*) doesn't provide ages.
20. Mysticeti	20.43	33.9	SB	*Morenocetus parvus*	*Morenocetus parvus* is the oldest described species belonging to crown-group Mysticeti, and is from the Aquitanian (minimum age = 20.43 Ma) in the early Miocene of Patagonia (*121*). There is also an unnamed balaenid (~28 Ma) from the late Oligocene of New Zealand (Kokoamu Greensand) (*122*), but the phylogenetic position of this taxon is unclear and awaits phylogenetic analysis (J. Geisler, communication to J. Gatesy).
21. Bovinae to Antilopinae	16	28.1	SB	*Pseudoeotragus*	*Pseudoeotragus* is a stem antelopine and is known from the Burdigalian (*78*).
22. Haplorrhini (=Tarsiidae to Anthropoidea)	38.0	59.2	PU	Numerous eosimiids	*Altiatlasius* is know from the late Paleocene and is a stem anthropoid in recent cladistic analyses (*123, 124*), although not in Bloch *et al.* (*125*). *Anthrasimias* (54-55 Ma, Sparnacian) (*123, 126*) is another putative stem anthropoid, although its anthropoid status has been questioned (*124*). *Algeripithecus* is younger (> 45 Ma) and has been referred to Anthropoidea (*127*). However, recent discoveries of more complete specimens of *Algeripithecus* suggest that it is not anthropoid (*128*). Instead, it may belong to the Azibiidae, which are argued to be adapiforms, early euprimates, plesiadapiforms (possible stem

					primates), or even nonprimates (*128*). As many as 11 species and six genera of Asian eosimiids are known from the middle Eocene of Asia (*128*). The oldest tarsiid is *Tarsius eocaenus* (*129, 130*) from the middle Eocene of China (minimum age = 38.0 Ma). We used the top of the middle Eocene as a minimum for crown Haplorhini based on the occurrence of numerous eosimiids from the middle Eocene (*128*). The maximum age allows for the possibility that the late Paleocene *Altiatlasius* from Morocco is a stem anthropoid (*128*).
23. Anthropoidea	28.1	56.0	PU	*Aegyptopithecus*	*Aegyptopithecus* (early Oligocene~Rupelian) is a stem catarrhine in Kay *et al.*'s (*131*) cladistic analyses with molecular scaffolds. The oldest stem platyrrhine is *Branisella* (*131*) from the late Oligocene of Bolivia (Salla Beds, Chron 8, age = 25.82-27.02 Ma) (*124*). Some authors have suggested that *Catapithecus browni* from the Priabonian is a stem catarrhine, but other authors (*e.g.*, Williams *et al.*; *128*) have suggested a stem anthropoid position for this taxon. Seiffert *et al.* (*127*) recovered a polytomy with catarrhines, platyrrhines, Parapithecoidea, Proteopithecidae, and *Algeripithecus minutus*. The age of the latter taxon is Ypresian to basal Lutetian, which established a maximum of 56.0 Ma for the base of Anthropoidea if we allow for phylogenetic uncertainty, *i.e.*, that *Algeripithecus* is either a crown anthropoid or one of the first two outgroups. In contrast, Tabuce *et al.* (*132*) place *Algeripithecus* as a stem strepsirrhine based on more complete material.
24. Catarrhini	20.55	38.0	PB/PU	*Afropithecus*	*Afropithecus turkanensis*, previously *Morotopithecus bishop* (*133*), is dated at 20.6 +/- 0.05 = 20.55 Ma based on overlying lava (*134*) and is the oldest crown-group catarrhine. It is a stem hominoid (*135*) or even a crown hominoid (*134*). The oldest cercopithecoid is *Victoriapithecus* (*136*) at ~19 Ma. Oligopithecids (including *Catopitheus*) are sometimes regarded as stem catarrhines (*e.g.*, see review of alternate hypotheses in Seiffert and Simons; *137*) where *Catopithecus* is sometimes part of second outgroup to crown catarrhines). The age of *Catopithecus* is 34.8-33.7 Ma (Chron C13r) based on Seiffert (*138*), which is mostly within the Priabonian. We used the base of the Priabonian (38.0 Ma) as the maximum age for the base of Catarrhini.
25. Hominoidea	11.608	28.1	SB	*Sivapithecus*	*Sivapithecus* is the oldest crown hominoid in Finarelli and Clyde's (*135*) cladistic and stratocladistic analyses.
26. Strepsirrhini	38.0	56	SB/PB	*Saharagalago*	*Saharagalago misrensis* is known from the Bartonian (*139*) and is assigned to Galagidae; *Karanisia* is as a crown lorisid in Seiffert *et al.*'s (*139*) analysis, although these authors also allow for the possibility that this taxon is a stem lorisid or stem lorisiform. These are the oldest crown strepsirrhines. Adapiforms are paraphyletic at the base of crown Strepsirrhini (see *140*). The oldest adapiforms are early Eocene and establish a maximum age for Strepsirrhini.
27. Chinchillidae to Octodontidae	24.5	38.0	SB/PB	*Sallamys, Xylechimys, Deseadomys*	Molecular studies (*141, 142*) suggest that *Myocastor*, and possibly *Capromys*, are nested within Echimyidae. The fossil taxa *Sallamys*, *Xylechimys*, and *Deseadomys* (family Echimyidae) are all known from the Deseadean (*143*), which is mostly late Oligocene and has a minimum age of 24.5 Ma (*144, 145*). These fossils establish the minimum age for Echimyidae (inclusive of *Capromys* and *Myocastor*). *Eosallamys, Eoespina, Eosachacui*, and *Eodelphomys* were assigned to Echimyidae and are known from the Santa Rosa Local Fauna of Peru (*146, 147*). The putative age of this fauna is late Eocene, but this age is based on stage of evolution arguments and remains to be firmly established using more rigorous stratigraphic methods. Hence, we incorporate this information into our maximum age but not our minimum age.
28. Phiomorpha + Caviomorpha	40.94	56	SB/PB	Stem caviomorphs in Antoine et al. (*146*) including *Cachiyacuy* and other stem Caviomorpha	This calibration was suggested by Phillips (*94*) based on fossils that were described by Antoine *et al.* (*148*) including *Cachiyacuy contamanensis*. We retained minimum and maximum ages suggested by Phillips (*94*). Hartenberger (*149*) contends that specimens of the early Eocene *Glibia* are instead the oldest representatives of

					Phiomyidae. Given this possibility we used the base of the early Eocene as the maximum age for Phiomorpha to Caviomorpha.
29. Myomorpha (=Muroidea to Dipodidae)	45	59.2	SB	*Pappocricetodon*	Eocene deposits in China with *Pappocricetodon* have been biocorrelated with Bridgerian and early Uintan LMAs of North America (*130*), and suggest an age of ~45 Ma. SB yields a maximum age of 59.2 Ma for Muroidea + Dipodidae.
30. *Mus* to *Rattus*	10.4	15.97		*Karnimata*	*Karnimata darwini* is known from the Dhok Pathan Formation, which has an absolute age in the vicinity of 10.4 Ma (*78*) within the Tortonian stage. *K. darwini* is closer to *Rattus* than *Mus* (*78*). We used 10.4 Ma as the minimum age for *Rattus* to *Mus* following Benton *et al.* (*78*). Benton *et al.* (*78*) suggested a maximum constraint of 14.0 Ma based on the oldest record of *Antemurus*, which is found in the Langhian stage. We used the base of the Langhian stage (15.97 Ma) rather than 14.0 Ma for the maximum age of *Mus* to *Rattus*.
31. Rodentia	56.0	66.0	SB	*Acritoparamys, Paramys*	The earliest crown rodents (*e.g.*, *Acritoparamys*, *Paramys*) are late Thanetian in age (*78*) and establish a minimum age of 56.0 Ma for Rodentia. Given this age, stratigraphic bounding supports a maximum age of 66.0 Ma, which agrees with Benton *et al.* (*78*).
32. Lagomorpha	47.6	61.6	SB/PB	Ypresian tarsals	Tarsals from the Vastan mine, India, belong to crown Lagomorpha in phylogenetic analyses (*150*; p. 1205). The age of the Vastan mine deposits is Ypresian (minimum = 47.6 Ma). The first and second outgroups to crown Lagomorpha are *Palaeolagus* and *Mimotona* (*151, 152*). The latter is from the late Paleocene, which includes the Selandian and Thanetian. *Dawsonolagus* (*153*) is another stem lagomorph, but has not been included in cladistic analyses.
33. Mammalia	160.7	227	PB/PU	*Ambondro*	*Ambondro*, from the Bathonian, is a crown mammal in a variety of analyses (*e.g.*, Lou *et al.* (*154*) and Woodburne *et al.* (*155*); reviewed in Benton *et al.* (*78*)), although its affinities with monotremes versus therians remain controversial. We followed Benton *et al.* (*78*) and used the top of the Bathonian, *i.e.*, 166.1 +/- 1.2 Ma = 164.9 Ma. Multituberculata are generally placed inside of crown Mammalia (*e.g.*, Kielan-Jaworowska *et al.; 156*). Haramiyids, in turn, may be related to multituberculates (*156-158*). The oldest haramiyid is *Thomasia* from the Norian (*159*). The oldest mammal fossils are *Adelobasileus* (*159*), *Gondwanadon* (*160*), and *Tikitherium* (*160*) from the Carnian, but there is no suggestion that any of these taxa belong to crown Mammalia. Given the uncertainty associated with the placement of Haramiyidae, including *Thomasia*, we employed a maximum of 227 Ma (base of Norian).
34. Neognathae	66	86.8	SB	*Vegavis iaai*	Clarke *et al.* (*161*); Benton *et al.* (*78, 118*)
35. Amniota	318	332.9	SB	*Hylonomus lyelli*	*Hylonomus* is from the Joggins Formation of Nova Scotia. The most recent estimates for the age of the Joggins Formation are in the range of 318-319 Ma (*78*) (based on Gradstein *et al.; 162*). The maximum age is based on the absence of crown amniotes in well-sampled, fossiliferous deposits that occur below the strata with *Hylonomus* (*78*).
36. Archosauria to Lepidosauria	255.9	295.9	SB	*Protorasaurus speneri*	*Protorasaurus speneri* is the oldest archosauromorph and has a minimum age estimate of 255.9 Ma (*78, 118*), which falls within the Wuchiapingian stage of the Permian. Benton *et al.* (*78*) suggested a maximum age of 295.9 Ma.
37. Tetrapoda	337	351	SB	*Lethiscus stocki*	*Lethiscus* is the oldest reptilomorph (stem amniote) and is from the Wardie Shales, which belong to the Lower Oil Shale Group. The age of the Wardie Shales is ~343.5-337 Ma (*78*) (based on Gradstein *et al.*; *162*). The maximum age for Tetrapoda is from Benton *et al.* (*78*).

[A] Table modified and updated after Meredith *et al*. (*46*). Abbreviations: PB, phylogenetic bracketing; PU, phylogenetic uncertainty; SB, stratigraphic bounding; Ma, million years.