Supplementary Materials for

Genomic basis for skin phenotype and cold adaptation in the extinct Steller’s sea cow

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SM1: Samples, data processing, and ancient DNA authentication

Provenance

1) Fossil collection

Dugong: Tissue sample M.41683.002 was transferred from the Australian Museum to the Swedish Museum of Natural History on 2 May 2017. Both institutions are entitled to the exemption provided by Article VII, paragraph 6, of the CITES Convention.

_Hydromalis_ bone fragments for all samples other than SNMB N51667 were collected from large ribs deposited on the beaches of the Commander Islands, Russia. Co-author Alexander Burdin collected these samples during paleontological expeditions in 2006 and 2007 by drilling small fragments from several dozen large ribs, leaving the ribs on the beaches so as to preserve them _in situ_. No permits were required either for within-country collection or for transfer of subsampled bone powder to the UCSC Paleogenomics Laboratory.

Sample SNMBN51667 was donated to the Braunschweig Natural History Museum in 1910 by Adolph Dattan, a resident of Vladyvostok, Russia. At that time, there was no regulation concerning trade of fossil bones. The specimen has been on public display in the Braunschweig Museum and described previously in several scientific publications.

2) Species-level validation and Radiocarbon Dating

Molecular species designation and radiocarbon dating of fossil fragments of _Hydromalis_ is described below and in the main text. Briefly, authenticity was verified by mapping read fragments to the genome of the dugong using mapping parameters described in what follows. Radiocarbon dates were obtained from each individual fragment at the W. M. Keck Carbon Cycle Accelerator Mass Spectrometry facility at UC Irvine and the Klaus-Tschira-AMS facility in the Curt-Engelhorn-Centre of Archaeometry in Mannheim, Germany.

3) Accessibility

Dugong M.41683.002 is part of the collection at the Australian Museum, 1 William St., Sydney, NSW 2010, Australia. _Hydromalis_ SNMBN51667 is part of the permanent collection of the Braunschweig Natural History Museum. Subsamples of _Hydromalis_ ribs were deliberately extremely small and, in most instances, were consumed in their entirety for the purposes of genomic analysis. In some cases, small amounts of bone material remains, and are accessioned at the UCSC Paleogenomics Lab with accession details provided in Table S1. When no material remains, access to residual DNA extracts or libraries can be requested from co-author Shapiro.

CT-scanning of the skull of a museum specimen Steller's sea cow (_Hydrodamalis gigas_) – SNMB N51667

To identify the most densely ossified region (MDOR), we performed a radiological assessment of the intact Steller’s sea cow skull with a dimension of 490 mm x 330 mm x 220 mm. We used a clinical 320-slice CT volume scanner Aquilion ONE from Toshiba Medical, Japan to scan the entire skull in 0.5 mm steps for a total of 2,721 slices. The scan parameters applied were as follows: 100 kV and 120 kV at 300 mA with a rotation time of 0.75 sec per revolution. We then used the reconstruction cores FC08, FC30, FC35, and FC81. We performed
post-processing for image analysis, including calculation of bone density and a complete 3D reconstruction using a VITREA Advanced Visualization Workstation (version 7.11.5.29) and a MacPro with DICOM Viewer Osirix (MD). After identification of the MDOR in the 3D reconstruction, we calculated and printed a virtual biopsy plan for drilling, so as to minimize destruction of the important museum specimen. In total we collected 1.0 g of bone by drilling.

Radiocarbon dating

Sample SNMB N51667 was radiocarbon dated at the Klaus-Tschira-AMS facility in the Curt-Engelhorn-Centre of Archaeometry in Mannheim, Germany (MAMS; laboratory number GMP 391). All other samples were radiocarbon dated at the Keck AMS laboratory at the University of California Irvine (KCCAMS). The methods were similar for all samples. For $^{14}$C and stable isotope determinations we followed methods described in Shammas et al. (32). Briefly, aliquots of ~200 mg of cortical bone were crushed to mm-sized chips and decalcified overnight with 1N HCl at room temperature, using a measured amount of acid calculated as just sufficient to dissolve all of the bone mineral if no collagen was present. The samples were then washed with ultrapure MQ water, gelatinized at pH 2 at 60°C overnight, and ultrafiltered with precleaned Vivaspin 15 Turbo ultrafiltration devices to select the >30 kDa molecular weight fraction, which was frozen and lyophilized overnight in a vacuum centrifuge.

2 mg aliquots of the lyophilized collagen for $^{14}$C dating were transferred to 6 mm quartz combustion tubes. CuO oxidizer and silver wire getter were added and the tubes were sealed under vacuum and combusted at 900°C. CO$_2$ graphitization and $^{14}$C measurement by AMS were carried out at the Keck AMS laboratory at the University of California Irvine. Aliquots containing 0.7 mg of collagen were wrapped in 5 x 9 mm tin foil cups and elemental analyses and $\delta^{13}$C and $\delta^{15}$N measurements were carried out using a Fisons NA1500NC elemental analyzer interfaced to a Finnigan Delta Plus isotope ratio mass spectrometer.

Two samples and a $^{14}$C-free blank were submitted as bone powder that had been partially pretreated for DNA studies with one or two 24-hour incubations with EDTA and proteinase K. These samples were washed 4 times with MQ water with vortexing, soaked overnight in MQ water, and washed 3 more times with MQ water, to remove residual EDTA and proteinase prior to the normal processing. Following the gelatinization step, we observed that some residual bone powder was present for one sample (SC16.JK045) and the blank, that had received one 24-hour incubation rather than two. Aliquots of this residual material were run as new samples using the protocol outlined above, and both returned ages were consistent with the results from the initial gelatinization.

Data processing

Base calling was done with Bustard (Illumina). We trimmed the adapters and merged overlapping paired-end reads into single sequences using leeHom (43). We aligned the reads to the de novo assembled dugong (SI2) and the manatee genomes (UCSC GCA_000243295.1 (9)) using the Burrows–Wheeler aligner (BWA, version 0.5.10-evan.9–1-g44db244) (45) with parameters adjusted for ancient DNA (-n 0.01 –o 2 –l 16500) (46). Reads were also aligned to the $H$. gigas mitochondrial genome (8). We removed PCR duplicates with bam-rmdup (version 0.6.3) and filtered fragments for read length ($\geq$ 32 bp) and mapping quality (MQ $\geq$ 30) using SAMtools (version 1.3.1) (44). We used the ancient DNA genotyping software snpAD (47) on scaffolds $\geq$ 100 kbp to infer per scaffold polymorphic positions in the twelve sequenced individuals.
For details on specimens used, data processing, and dating refer to Supplementary Table S1.

Authentication of ancient DNA

A characteristic of ancient DNA is that cytosines (C) located in proximity of DNA fragment ends undergo deamination to uracils. Thus, in the process of library preparation, DNA polymerases incorporate thymine (T) instead of C. We evaluated the C to T substitutions to authenticate the presence of ancient DNA in our sequences. The read-length distribution and substitution rates at the 5’ and 3’ ends in our data set (Supplementary Fig. S1) are in accordance with high fragmentation and modification of ancient DNA, respectively.
**Supplementary Table S1.**

List of used specimens (museum catalog number), sequencing stats, and radiocarbon dating. MQ30 = mapping quality >30, UDG= uracil-DNA glycosylase, UCSC=University of California Santa Cruz.

| ID      | Location Processed | Library Prep Method | UDG Treated Extract | No. Libraries | Raw Reads  | Reads Passing Filters (Merged, Mapped, Length > 32, MQ > 30) | % Reads Mapping | Average Duplicates | Average Read Length MQ30 (bp) | Average Fold Coverage | 
|---------|-------------------|---------------------|---------------------|---------------|------------|---------------------------------------------------------------|----------------|------------------|----------------------------|---------------------|  
| SC16.JK043 | UCSC              | Kapp, 2021           | N                   | 8             | 426,518,193 | 151,963,285                                                  | 41.31          | 1.25             | 58                        | 2.28                | 1,380±20       
| SC16.JK044 | UCSC              | Kapp, 2021           | N                   | 6             | 618,500,715 | 112,533,135                                                  | 21.19          | 1.23             | 67                        | 1.97                | 1,250±15       
| SC16.JK052 | UCSC              | Kapp, 2021           | N                   | 6             | 501,237,072 | 115,212,168                                                  | 27.88          | 1.26             | 73                        | 2.14                | 1,245±15       
| SC16.JK057 | UCSC              | Kapp, 2021           | N                   | 6             | 478,650,735 | 120,501,949                                                  | 26.37          | 1.27             | 75                        | 2.28                | 1300±15        
| SC16.JK061 | UCSC              | Kapp, 2021           | N                   | 8             | 664,459,501 | 243,889,647                                                  | 42.89          | 1.26             | 61                        | 3.78                | N/A            
| SC16.JK064 | UCSC              | Kapp, 2021           | N                   | 8             | 727,872,124 | 188,646,115                                                  | 29.79          | 1.27             | 71                        | 3.38                | 1,565±15       
| SC16.JK066 | UCSC              | Kapp, 2021           | N                   | 10            | 770,625,574 | 228,951,014                                                  | 33.67          | 1.26             | 68                        | 3.95                | 2,205±15       
| SC16.JK069 | UCSC              | Kapp, 2021           | N                   | 6             | 641,996,782 | 155,730,543                                                  | 28.89          | 1.24             | 65                        | 2.58                | 1,155±20       
| SC16.JK070 | UCSC              | Kapp, 2021           | N                   | 6             | 599,236,387 | 170,509,149                                                  | 33.76          | 1.26             | 62                        | 2.69                | 1,250±15       
| SC16.JK076 | UCSC              | Kapp, 2021           | N                   | 4             | 526,920,529 | 170,200,152                                                  | 36.59          | 1.26             | 64                        | 2.77                | 1,300±15       
| SNMB N51667 | University of Potsdam | Gansauge & Meyer, 2013 | Y                   | 3             | 10,896,135,224 | 1,152,095,440                                                  | 15.4           | 1.07             | 46                        | 15.86                | 1,267±19       
| SC16.JK045 | UCSC              | Kapp, 2021           | N                   | 12            | 2,613,737,582 | 1,007,518,366                                                  | 48.45          | 1.21             | 59                        | 15.63                | 1,260±15/1,255±15/1,270±20 |
Supplementary Fig. S1.

Analyses of genomic sequences from the extinct Steller’s sea cow individuals. Read-length distribution for sample SNMB N51667 of all reads (black curve) and reads mapped to the dugong genome without a mapping quality threshold (red) show a high fragmentation rate. The substitution patterns from the 5’ and 3’ ends of the sequenced reads indicate that the mapped reads are of ancient origin. Sample SNMB N51667 was treated with uracil-DNA glycosylase and endonuclease VIII prior to library preparation, effectively reducing DNA damage as shown by the lower C>T substitution frequencies compared to the other samples.
Supplementary Fig. S1 – continued.

Analyses of genomic sequences from the extinct Steller’s sea cow individuals. The substitution patterns from the 5’ and 3’ ends of the sequenced reads indicate that the mapped reads are of ancient origin.
Pairwise distances between sequenced individuals based on mitochondrial genomes

To test whether the 12 sequenced fossils originated from different individuals, we estimated pairwise differences based on their mitochondrial genome sequences. For each sample we called genotypes using samtools mpileup (44) on reads mapped to the Steller’s sea cow mitochondrial genome (8). In the further analyses we included positions with MQ ≥ 30, GQ ≥ 20, and coverage ≥ 10 in all samples. For coverage and read depth statistics refer to Supplementary Table S2. Finally, we created a pairwise distance matrix using the number of polymorphisms between samples. Based on pairwise distances we concluded that samples originated from different individuals (Supplementary Fig. S2).

Supplementary Table S2.

Coverage of the mitochondrial genomes based on reads with mapping quality ≥ 30, genotype quality ≥ 20, and coverage ≥ 10.

| Sample ID    | Mean depth | No. covered bases | Coverage of mitochondrial genome (%) |
|--------------|------------|-------------------|--------------------------------------|
| SC16.JK045   | 2,994      | 16,306            | 95.5                                 |
| SNMB N51667  | 1,349      | 16,165            | 94.7                                 |
| SC16.JK043   | 886        | 16,156            | 94.6                                 |
| SC16.JK044   | 236        | 15,833            | 92.7                                 |
| SC16.JK052   | 863        | 16,117            | 94.4                                 |
| SC16.JK057   | 1127       | 16,173            | 94.7                                 |
| SC16.JK061   | 616        | 16,043            | 93.9                                 |
| SC16.JK064   | 1,037      | 16,119            | 94.4                                 |
| SC16.JK066   | 2,007      | 16,220            | 95.0                                 |
| SC16.JK069   | 276        | 15,859            | 92.9                                 |
| SC16.JK070   | 221        | 16,014            | 93.8                                 |
| SC16.JK076   | 444        | 15,951            | 93.4                                 |
Supplementary Fig. S2.

Box plots of the pairwise distances per sample. Numbers on the bars represent the $^{14}$C uncalibrated date (years BP). The upper whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Data beyond the end of the whiskers are "outlying" points plotted individually.
**SM2: Dugong dugon genome**

*De novo assembly*

The final *Dugong dugon* assembly is 3,196.6 Mbp in length with a scaffold N50 contiguity of 3110.4 kbp (Supplementary Table S3). The BUSCO (39) assessment using 303 eukaryotic orthologs (not shown) found that the additional ARKS+LINKS scaffolding did not disturb the gene completeness of the assembly, but rather decreased the missing gene count and also the duplicate count by a factor of 2.

The BUSCO evaluation of mammalian single-copy orthologs shows the final assembly to be highly gene complete, with few haplotig artefacts: C:93.0% [S:92.1%, D:0.9%], F:4.2%, M:2.8%, n:4104.

**Supplementary Table S3.**

Assembly metrics of the dugong genome. bp = base pairs.

| Metric                              | Value          |
|-------------------------------------|----------------|
| Total number of scaffolds           | 16,045         |
| Total number of bases (Mbp)         | 3,196.9        |
| N50 (Mbp)                           | 3.11           |
| Number of scaffolds > 1 kbp – 10 kbp | 16,063 (100 %) |
| Number of scaffolds >10 kbp – 100 kbp | 4,779 (29.7 %) |
| Number of scaffolds > 100 kbp – 1 Mbp | 2,114 (13.2 %) |
| Number of scaffolds > 1 Mbp         | 832 (5.2 %)    |
| Total number of contigs             | 40,386         |
| Number of contigs in scaffolds      | 27,820         |
| Number of contigs not in scaffolds  | 12,566         |
| Percentage of assembly in scaffolded contigs | 96.8 % |
| Percentage of assembly in un-scaffolded contigs | 3.2 % |

**Annotation of the dugong and manatee genomes**

We used MAKER (10) to annotate the dugong (*Dugong dugon*) and manatee (*Trichechus manatus latirostris* (9); UCSC GCA_000243295.1) genomes. We included several sources of evidence in the annotation process: *de novo* gene prediction using SNAP (52) and protein sequences of three high-quality genomes (human – GRCh38.p13, mouse – GRCm38.p6, and elephant – Loxafr3.0) downloaded from Ensembl v98 (11). We used RepeatMasker v4.1.0 (53) to identify repeats that matched to entries in standard databases for known repetitive sequences. To increase the likelihood of identifying coding regions, we also used known proteins to identify homologous sequences in our assembly. BLASTX (54) was used to align protein data against the raw genomic sequence; if the alignment occurred within the same coordinates with the *de novo* prediction, we considered the region homologous. We applied a threshold of 300 bp for the *ab initio* predicted genes; with additional evidence informed gene prediction, we obtained consensus gene sets of 36,749 and 25,192 genes for dugong and manatee, respectively.

**Orthology assignment**
Starting from human annotations, orthologs between human, mouse, and elephant were downloaded from Ensembl v98 (11). Dugong genes for which the MAKER annotation ortholog was in accordance with the triplet downloaded from Ensembl were included in further analysis. Orthologs transitivity is a major challenge in accurately assigning ortholog groups among multiple species. We also considered only genes that agreed with the orthologous triplets for the manatee. Predicted coding sequences of manatee and dugong were aligned with TBLASTX (54) and we filtered only the reciprocal best hits with an e-value $\leq 10^{-10}$. We obtained 4,877 1:1 orthologous genes in the five species (please find here Supplementary Table S4).

SM3: Comparative genomic analyses

We then compared the Steller’s sea cow genome to the genomes of extant sirenians – dugong and manatee – and three additional well-characterized genomes: human, mouse, and elephant.

Inference of the Steller’s sea cow coding sequence

To identify changes that occurred on the Steller’s sea cow lineage, we called variants on reads aligned to the dugong genome using snpAD (47). Based on the set of 4,877 1:1 orthologous genes in dugong, manatee, human, mouse, and elephant, we identified the coordinates of the coding sequences in dugong. We lifted those coordinates from the variant called files in the genome of SNMB N51667, since this library showed the lowest level of damage (Supplementary Fig. S1) and, thus, posed the lowest risk of introducing erroneous mutations. We focused only on positions with coverage $\geq 5\times$ and GQ $\geq 20$; all other positions were deemed as unresolved and replaced by “N”s (Supplementary Table S4). Variants in manually curated genes were inquired in all individuals.

The six-species orthologs were subjected to codon-based multiple alignments using MACSE v2 (48). Regions containing gaps in any of the species were excluded.

Inactivated genes

As pseudogenization is one means of shaping phenotypes, we screened the orthologs for premature stop codons. We manually curated the identified genes by inspecting the multiple sequence alignments for the presence of stop codons in all sequenced individuals. For this purpose, we did not filter the called variants for any quality criteria in the low-coverage individuals and used the number of supporting reads to calculate the probability of error based on the ancient DNA deamination pattern. We identified 10 genes with premature stop codons on the Steller’s sea cow lineage (Supplementary Table S5).

For variants that implied C>T or G>A changes, we visualized the reads to exclude that the change occurred at the fragment ends. Furthermore, we calculated the probability that the variant is an error (Supplementary Table S6) by considering the number of reads and the highest error frequency at read ends, i.e. 0.2 (Supplementary Fig. S1). We used Bayes’ theorem for each individual as follows:
\[ P(\text{variant|error} = P(\text{error|variant} \cdot P(\text{variant}) \frac{P(\text{variant})}{P(\text{error})}, \text{ where } P(\text{error|variant} = 0.2, P(\text{variant}) = 1 - 0.25^{\text{no.of reads with variant}}, \text{ and } P(\text{error}) = 0.25). \] The posterior probability of error was calculated per individual as \(1 - P(\text{error}) = 0.25\) and the final probability of error was the product of all individual error probabilities (Supplementary Table S6).

For gene ontology enrichment analyses of inactivated genes along the lineage to Steller’s sea cow, we downloaded the Gene Ontology (GO) annotation from Ensembl v98 (11) and tested for enrichment using the FUNC package (55). We ran a hypergeometric test in which the inactivated genes were the set of interest and the rest of the 4,877 annotated 1:1 orthologs were the background. The analysis identified GO categories related to skin function (Supplementary Table S7), which include two arachidonate lipoxygenases (ALOX3 and ALOX12B).

**Supplementary Table S5.**

Genes with premature stop codons in Steller’s sea cow. The location of the stop codon is given based on the human canonical transcript and the allele frequency in the human population is given based on the genome aggregation database (https://gnomad.broadinstitute.org). * for CDH24 two bases in the same codon are different in Steller’s sea cow compared to the human transcript.

| Gene name | Gene description | Premature stop codon (corresponding to human transcript) | Protein length aa (human) | Variation in human population AF (GnomAD) |
|-----------|------------------|--------------------------------------------------------|---------------------------|----------------------------------------|
| ALOX12B   | arachidonate 12-  | NM_001139.3:c.312G>A, p.(Trp104*)                      | 701                       | 0                                      |
|           | lipooxygenase, 12R| type                                                  |                           |                                        |
| ALOXE3    | arachidonate lipoxygenase | NM_001165960.1:c.1578G>A, p.(Trp526*) | 711                       | 0                                      |
|           | 3                |                                                        |                           |                                        |
| CDH24*    | cadherin 24      | NM_022478.3:c.382_384delCGGinsTGA, p.(Arg128*)         | 819                       | 0                                      |
| EHHADH    | enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase | NM_001966.3:c.1674C>A, p.(Tyr558*) | 723                       | 0                                      |
| KRT36     | keratin 36       | NM_003771.4:c.376C>T, p.(Gln126*)                      | 467                       | 0                                      |
| NPFFR2    | neuropeptide FF receptor | NM_004885.2:c.1222G>T, p.(Glu408*)                       | 522                       | 0                                      |
| PAD2      | peptidyl arginine | NM_007365.2:c.1573C>T, p.(Arg525*)                      | 665                       | 7.9e-6                                 |
|           | deiminase 2      |                                                        |                           |                                        |
| SLC27A6   | solute carrier family 27 member 6 | NM_014031.3:c.1431G>A, p.(Trp477*)                       | 619                       | 3.9e06                                 |
| SLPI      | secretory leukocyte peptidase inhibitor | NM_003064.3:c.181A>T, p.(Lys61*)                   | 132                       | 0                                      |
| SMCO2     | single-pass membrane protein with coiled-coil domains 2 | NM_001145010.1:c.175A>T, p.(Arg59*)                       | 343                       | 0                                      |
Supplementary Table S6.

Genes with premature stop codons in Steller’s sea cow. For each individual we show the number of reads supporting the stop codon. All positions are homozygous in the sequenced individuals. For C>T or G>A changes we calculated the probability that the change is an error based on the total number of reads from all individuals and the highest error frequency observed at read ends, i.e. 0.2.

| Gene   | Position | Change | SC16.J K043 | SC16.J K044 | SC16.J K045 | SC16.J K052 | SC16.J K057 | SC16.J K061 | SC16.J K064 | SC16.J K066 | SC16.J K069 | SC16.J K070 | SC16.J K076 | SN MB S51 N67 | Probability of error |
|--------|----------|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------------|-----------------------|
| ALOXE3 | Scaffold d1250: 637,303 | G → A | 4 | 3 | 26 | 2 | 6 | 1 | 2 | 8 | 4 | 1 | 5 | 12 | 2.819e-08 |
| ALOX12B | Scaffold d1250: 661,569 | G → A | 1 | 5 | 26 | 4 | 9 | 9 | 7 | 8 | 3 | 8 | 3 | 16 | 9.432e-09 |
| NPF FR2 | Scaffold d41: 5,660,445 | T → A | 3 | 3 | 16 | 0 | 1 | 2 | 1 | 2 | 2 | 4 | 2 | 15 | |
| CDH24 | scaffold 1033: 1,142,036 | C → T | 2 | 8 | 32 | 5 | 5 | 9 | 10 | 4 | 5 | 3 | 0 | 9 | 2.795e-08 |
| KRT36 | scaffold 1341: 135,064 | C → T | 4 | 4 | 29 | 2 | 11 | 4 | 11 | 12 | 6 | 5 | 5 | 13 | 5.411e-09 |
| SMC02 | scaffold 288: 276,090 | C → T | 3 | 1 | 37 | 2 | 5 | 8 | 3 | 12 | 3 | 1 | 3 | 26 | 2.620e-08 |
| EHH ADH | scaffold 174: 953,143 | A → T | 3 | 0 | 27 | 4 | 1 | 6 | 2 | 5 | 4 | 7 | 5 | 25 | |
| PADI2 | scaffold 758: 738,091 | G → A | 1 | 0 | 23 | 0 | 1 | 3 | 6 | 1 | 2 | 1 | 4 | 10 | 2.212e-06 |
| SLC23A6 | scaffold 35: 3,227,356 | A → T | 5 | 3 | 18 | 0 | 2 | 3 | 1 | 1 | 3 | 1 | 2 | 19 | |
| SLPI | scaffold 956: 603,339 | T → A | 7 | 3 | 35 | 6 | 3 | 6 | 6 | 10 | 2 | 5 | 2 | 33 | |

Supplementary Table S7.

Gene ontology categories, which show enrichment with genes inactivated in Steller’s sea cow. Categories in bold are related to skin function. FDR=False discovery rate.

| Node name | Node ID | No. genes in node | No. significant genes in node | FDR | Significant genes in node |
|-----------|---------|-------------------|-------------------------------|------|--------------------------|
| oxidoreductase activity, acting on single donors with incorporation of molecular oxygen | GO:0016701 | 10 | 2 | 0.016971 | ALOXE3, ALOX12B |
| oxidoreductase activity, acting on single donors with incorporation of molecular | GO:0016702 | 9 | 2 | 0.020844 | ALOXE3, ALOX12B |
| Node name                                                                 | Node ID   | No. genes in node | No. significant genes in node | FDR     | Significant genes in node |
|--------------------------------------------------------------------------|-----------|-------------------|------------------------------|---------|--------------------------|
| oxygen, incorporation of two atoms of oxygen                             | GO:0016829| 69                | 3                            | 0.013267| ALOXE3, ALOX12B, EHHADH  |
| lyase activity                                                           | GO:0016853| 59                | 3                            | 0.012792| ALOXE3, ALOX12B, EHHADH  |
| isomerase activity                                                        | GO:0001676| 31                | 3                            | 0.011853| ALOXE3, ALOX12B, SLC27A6 |
| long-chain fatty acid metabolic process                                  | GO:006631 | 123               | 4                            | 0.017067| ALOXE3, ALOX12B, EHHADH  |
| fatty acid metabolic process                                             | GO:0019372| 8                 | 2                            | 0.02308 | ALOXE3, ALOX12B          |
| lipoxygenase pathway                                                      | GO:0033561| 11                | 2                            | 0.036389| ALOXE3, ALOX12B          |
| regulation of water loss via skin                                         | GO:0042759| 10                | 2                            | 0.03259 | ALOXE3, ALOX12B          |
| long-chain fatty acid biosynthetic process                               | GO:0043651| 6                 | 2                            | 0.01851 | ALOXE3, ALOX12B          |
| linoleic acid metabolic process                                           | GO:0051121| 3                 | 2                            | 0.007955| ALOXE3, ALOX12B          |
| hepoxilin metabolic process                                              | GO:0051122| 3                 | 2                            | 0.007955| ALOXE3, ALOX12B          |
| hepoxilin biosynthetic process                                           | GO:0061436| 8                 | 2                            | 0.02308 | ALOXE3, ALOX12B          |

**ALOX12B and ALOXE3**

The premature stop codons in *ALOX12B* and *ALOXE3* were present in all sequenced individuals (Supplementary Table S6). To infer the allele frequency in the population we used the jeffreysci function from the ratesci package in R (50). This allows a Bayesian inference of the allele frequency interval using the non-informative Jeffreys prior for a binomial distribution. The estimated allele frequency interval is 0.90–1 with a posterior probability of >0.95.

Furthermore, we inspected the *ALOX12B* and *ALOXE3* read alignments and identified a one-base insertion in *ALOX12B*, which leads to the following frameshift in the corresponding human canonical transcript: NM_001139.3: c.1350dup, p.(Leu451Ala fs*27). *ALOX3* also showed signals of faster evolution in Steller’s sea cow and cetaceans (Supplementary Table S8).

We therefore inspected the *ALOX12B* and *ALOXE3* sequences in all mammals, including cetaceans and pinnipeds, for which data are available. We used biomartr (56) to retrieve all mammalian coding sequences from refseq (accession date 13.04.2021), which we cross checked with the Ensembl v103 entries. Interestingly, in support of the results from Reisch and colleagues (21) we could also not find any *ALOX12B* for the available cetaceans, but the gene was intact in pinnipeds (Supplementary Fig. S3). Furthermore, we identified multiple inactivating mutations in *ALOX3* in cetaceans (Supplementary Fig. S4), while this gene was also intact in the pinnipeds.
Reisch and colleagues suggested that the complete loss of ALOX12B gene in cetaceans, but not in other marine, fur-bearing mammals, could be related to their lack of hair (21). The same was true for ALOXE3, although the pseudogene was still present in the cetacean genomes (21). The different skin phenotype of whales and dolphins compared to humans (16–18, 57–59) and mice (14, 19) with ALOX12B- and ALOXE3-gene loss was considered a potential evidence for different mechanisms involved in epidermal differentiation in marine mammals compared to their terrestrial counterparts (21, 60).

However, Steller described the skin of H. gigas to resemble “the bark of an old oak tree” and to be “extremely thick” (29), which appears to be in agreement with ichthyotic skin. We were thus prompted to understand what could prevent the formation of calluses especially in dolphins, which also lack the skin lipoxygenase genes (Supplementary Fig. S3 and S4). Cetaceans and particularly dolphins have a very high shedding rate of the stratum corneum of skin, which maintains a smooth surface and limits microbe colonization (22, 61). The outermost cell layer of dolphins can exfoliate up to 12 times a day (61). This high turnover rate ensures a smooth body surface and probably helps increase swimming efficiency by reducing drag (61). A potential molecular explanation for this observation is the lower number of cell structures specialized for cell-to-cell adhesions, called desmosomes, in the superficial layer of cetacean skin (22). Desmosomes are assembled from desmogleins (DSG) and desmocollins (DSC); e.g., the human genome encodes three DSC (DSC1–DSC3) and four DSG (DSG1–DSG4) proteins (23). The strongest-binding desmosomes occur in the outermost layers of human skin (DSG1:DSC1 and DSG4:DSC1) (23). Yet, cetaceans bear inactivations of DSC1 and DSG4, which prevents the formation of desmosomes and leads to a high exfoliation rate (22). Thus, even in the absence of skin lipoxygenase genes, cetaceans cannot develop a hyperkeratotic, scaling skin phenotype. However, sirenians, including Steller’s sea cow appear to have functional desmosomes (multiple sequence alignment can be downloaded here) and hence, Steller’s description might indeed have depicted an ichthyotic skin.

Our finding of convergent inactivation of skin lipoxygenase genes in cetaceans and Steller’s sea cow has several possible implications:

i. The inactivation of desmosome genes predated the ALOXE3 inactivation and it was thus believed that ALOXE3 loss is not adaptive, but rather its function is not needed as a result of adaptation through other mechanisms (22). However, the inactivation in Steller’s sea cow in the presence of functional desmosomes challenges this hypothesis.

ii. ALOX12B and ALOXE3 inactivations in cetaceans have been related to the skin innovations needed at the transition from land to water. The presence of a functional copy in pinnipeds initially suggested that these gene losses may be related to adaptation to a fully aquatic environment. However, we now show that extant sirenians, which are also fully aquatic, have a functional copy of both genes. The loss of the two genes in Steller’s sea cow, the only cold adapted sirenian, suggests that these genes could play a role in adaptation to a cold marine environment.

iii. While ALOX12B appears to be completely lost from cetacean genomes, ALOXE3 suffered independent inactivations on this lineage (22), as well as on the Steller’s sea cow’s branch (Supplementary Fig. S4). It is thus possible that the inactivation of ALOX12B preceded the one of ALOXE3; ALOX12B is located upstream of ALOXE3 in the cascade pathway for epidermic structural lipid formation (14). Since this pathway
became non-functional, *ALOXE3* could be drifting, which may explain the multiple inactivations in the different cetacean lineages. While we cannot clearly identify the functional relevance of these inactivations, their convergent molecular evolution suggests a role in the adaptation to cold aquatic life. Steller’s sea cow genomic data will thus be an important resource for further delineating signals of parallel evolution and their importance for adaptation to their environment.
Supplementary Fig. S3.

Phylogeny of 97 species for which we inspected the coding sequence of \textit{ALOX12B} (multiple sequence alignment can be downloaded \textcolor{red}{here}). We could not identify any sequence for cetaceans in the mammalian coding sequences we downloaded from refseq (accession date 13.04.2021) using biomartr (56, 62, 63) and cross checked with the Ensembl v103 entries. \textit{ALOX12B} is inactivated in Steller’s sea cow marked in red, while all other inquired species have a functional copy. The convergent inactivation in cetaceans and Steller’s sea cow suggests a role in the adaptation to cold marine environments.
Supplementary Fig. S4.

Phylogeny of 109 species for which we inspected the coding sequence of *ALOXE3* (downloaded from refseq using biomart (56, 62, 63) and cross checked with the Ensembl v103 entries – accession date 13.04.2021; multiple sequence alignment can be downloaded here). There are multiple independent inactivation events (in red) in cetaceans and in Steller’s sea cow, showing parallel evolution and suggesting a role in the adaptation to cold marine environments.
We further identified a premature stop codon in NPFFR2 (scaffold41: 5660445 T>A), which was confirmed in 11 of 12 sequenced individuals (SC16.JK052 had zero reads at this position, Supplementary Table S6), but also in the assembly from Sharko and colleagues (15). Based on our 12 sequenced individuals, the estimated allele frequency is 0.91 (range 0.76–0.98 with a 0.95 confidence level).

To detect whether NPFFR2 occurs specifically on Steller's sea cow branch, we inspected all mammalian coding sequences we could retrieve from refseq using biomart (56) (accession date 13.04.2021; multiple sequence alignment can be downloaded here). Interestingly, we found that NPFFR2 has also been inactivated multiple times independently in cetaceans, where only the blue whale (Balaenoptera musculus) appears to still have an intact ortholog (Supplementary Fig. S5). The single leucine deletions in the different whale and dolphin linages affect positions 100% conserved in all other mammals. Furthermore, L334 and L395 are part of the transmembrane alpha-helices 5 and 6, respectively. Their loss leads to a change in the helical periodicity and, therefore, to a nonfunctional rearrangement of the transmembrane helix bundle. NPFFR2 is also pseudogenized in the Indian flying fox (Pteropus giganteus, Supplementary Fig. S5). Although from an evolutionary perspective we could not identify a common habitat adaptation with the Indian flying fox, the independent inactivation of NPFFR2 in multiple cetaceans, as well as in Steller’s sea cow, suggests that in an aquatic environment there may be a relaxed selection on this gene. However, the mouse Npffr2 knockout phenotype (24) could imply that this gene loss may be beneficial for a capital-based survival strategy, as in species which undergo seasonal fasting, much like the Steller’s sea cow.
Supplementary Fig. S5.
Phylogeny of 127 species for which we inspected the coding sequence of NPFFR2 (downloaded from refseq using biomart (56, 62, 63) – accession date 13.04.2021; multiple sequence alignment can be downloaded here). There are multiple independent inactivation events (in red) in cetaceans and in Steller’s sea cow, as well as in the Indian flying fox (Pteropus giganteus). The independent inactivations may suggest a relaxed selection in an aquatic environment.
Selection analysis

To examine selective constraints along the lineage to the Steller’s sea cow, we scanned for differently evolving genes with the CODEML program under a branch model (49) using previously curated orthologs in the six species (Steller’s sea cow, dugong, manatee, human, mouse, and elephant). We performed likelihood ratio tests (LRTs) to compare evolutionary models on our phylogenetic tree. First, we estimated an average $\omega$ across the tree using model = 0. We used the one-ratio model (model = 0, NSsites = 0) to estimate the same $\omega$ ratio for all branches in the phylogeny. We then used the two-ratio model (model = 2, NSsites = 0), with a background $\omega$ ratio and a different $\omega$ on the Steller’s sea cow lineage. These two models were compared via a LRT (1 degree of freedom) (49). We used a chi-square test to check whether the Steller’s sea cow branch is significantly different and if the estimated $\omega$ is higher or lower than for the other species, which would imply that these genes are differently evolving (faster or slower). This yielded 197 genes with a faster (Supplementary Table S8) and 41 genes with a slower evolutionary rate (Supplementary Table S9) in the Steller’s sea cow lineage compared to the other five species in the tree, which also included extant sirenians.

To identify genes, which could be relevant for aquatic adaptation we tested, using the setup described above and a phylogenetic tree including the same outgroup species (human, mouse, and elephant), 2 cetaceans (blue whale and bottlenose dolphin), and one pinniped (walrus), whether the identified genes show signals of selection on any of the aquatic-adapted branches.

Supplementary Table S8.

Genes with signs of faster evolution on the Steller’s sea cow branch. $P$-value calculated with a chi-square test based on the likelihood ratio test between model 0 and model 2 in CODEML (49) (1 degree of freedom). Faster evolving genes on the Steller’s sea cow lineage associated with energy homeostasis regulation and body weight are marked * (literature search via Pubmed, OMIM and Mouse Genome Informatics (http://www.informatics.jax.org)).

$^1$faster evolving in cetaceans; $^2$more slowly evolving in cetaceans; $^3$faster evolving in pinnipeds; $^4$more slowly evolving in pinnipeds.

| Gene name | Gene description | $\omega_{\text{model0}}$ | $\omega_{\text{background}}$ | $\omega_{\text{foreground branch}}$ | $p$-value |
|-----------|------------------|--------------------------|-----------------------------|-------------------------------------|-----------|
| $AAMP$    | angio associated migratory cell protein | 0.02                   | 0.02                        | 0.39                               | $1.24e-04$ |
| $ACP4^1$ | acid phosphatase 4 | 0.17                   | 0.16                        | 1.51                               | $7.84e-03$ |
| $ACP6$    | acid phosphatase 6, lysophosphatidic | 0.33                   | 0.31                        | 2.38                               | $2.45e-02$ |
| $ACP7^1$ | acid phosphatase 7, tartrate resistant (putative) | 0.11                   | 0.1                         | 0.65                               | $7.33e-03$ |
| $ACSF3^*$ | acyl-CoA synthetase family member 3 | 0.18                   | 0.17                        | 2.31                               | $2.45e-03$ |
| $ACTBL2^1$ | actin beta like 2 | 0.09                   | 0.08                        | 0.73                               | $1.17e-02$ |
| Gene name     | Gene description                                      | $\omega_{\text{model}}$ | $\omega_{\text{background}}$ | $\omega_{\text{foreground branch}}$ | $p$-value       |
|---------------|-------------------------------------------------------|--------------------------|-------------------------------|--------------------------------------|-----------------|
| ADGRG7*       | adhesion G protein-coupled receptor G7                | 0.5                      | 0.48                          | 2.03                                 | 4.50e-02        |
| ALKBH1*       | alkB homolog 1, histone H2A dioxigenase               | 0.22                     | 0.21                          | 1.95                                 | 1.65e-02        |
| ALOXE3*       | arachidonate lipoxygenase 3                           | 0.12                     | 0.11                          | 1.1                                  | 5.67e-05        |
| ALS2CL*       | ALS2 C-terminal like                                  | 0.13                     | 0.13                          | 0.35                                 | 2.10e-02        |
| AMPD2*        | adenosine monophosphate deaminase 2                   | 0.03                     | 0.02                          | 0.1                                  | 3.43e-02        |
| APLP1         | amyloid beta precursor like protein 1                 | 0.2                      | 0.19                          | 1.08                                 | 1.97e-02        |
| APTX          | aprataxin                                             | 0.24                     | 0.23                          | 1.06                                 | 4.52e-02        |
| ARHGAP22      | Rho GTPase activating protein 22                      | 0.13                     | 0.12                          | 0.71                                 | 2.90e-02        |
| ASNSD1        | asparagine synthetase domain containing 1             | 0.24                     | 0.24                          | 1.53                                 | 4.83e-02        |
| ASPHD1        | aspartate beta-hydroxylase domain containing 1        | 0.11                     | 0.11                          | 1.27                                 | 1.98e-02        |
| BCAS2         | BCAS2 pre-mRNA processing factor                      | 0.07                     | 0.04                          | 0.95                                 | 7.79e-03        |
| BCO1*         | beta-carotene oxygenase 1                             | 0.12                     | 0.12                          | 1.05                                 | 4.11e-02        |
| BMPRIA*       | bone morphogenetic protein receptor type 1A           | 0.01                     | 0.01                          | 0.37                                 | 4.07e-02        |
| BNIP1         | BCL2 interacting protein like                         | 0.19                     | 0.18                          | 0.93                                 | 1.71e-02        |
| BVES          | blood vessel epicardial substance                     | 0.16                     | 0.15                          | 0.96                                 | 2.22e-02        |
| Clorf116      | chromosome 1 open reading frame 116                  | 0.44                     | 0.43                          | 1.58                                 | 2.72e-02        |
| CA7           | carbonic anhydrase 7                                  | 0.1                      | 0.09                          | 0.49                                 | 2.23e-02        |
| CACNG1        | calcium voltage-gated channel auxiliary subunit gamma 1 | 0.11                    | 0.1                           | 0.94                                 | 3.47e-02        |
| CCDC137*      | coiled-coil domain containing 137                    | 0.15                     | 0.14                          | 1.22                                 | 3.93e-02        |
| CCT4*         | chaperonin containing TCP1 subunit 4                 | 0.04                     | 0.04                          | 0.24                                 | 2.52e-02        |
| CCT7          | chaperonin containing TCP1 subunit 7                  | 0.05                     | 0.04                          | 0.25                                 | 3.70e-02        |
| CDC23         | cell division cycle 23                                | 0.03                     | 0.02                          | 0.24                                 | 2.67e-03        |
| CDH24*        | cadherin 24                                           | 0.14                     | 0.13                          | 2.36                                 | 1.01e-02        |
| CDR2*         | cerebellar degeneration related protein 2             | 0.16                     | 0.16                          | 0.91                                 | 2.75e-02        |
| CFAP52*       | cilia and flagella associated protein 52              | 0.09                     | 0.09                          | 0.62                                 | 4.34e-02        |
| CLDN4         | claudin 4                                             | 0.07                     | 0.06                          | 0.52                                 | 4.47e-02        |
| Gene name | Gene description                                      | $\omega_{model0}$ | $\omega_{background}$ | $\omega_{foreground branch}$ | $p$-value   |
|-----------|------------------------------------------------------|-------------------|------------------------|-------------------------------|-------------|
| CMTR2     | cap methyltransferase 2                              | 0.19              | 0.18                   | 1.4                           | 3.83e-02    |
| CNDP1     | carnosine dipeptidase 1                              | 0.16              | 0.16                   | 0.66                          | 4.72e-02    |
| COG1      | component of oligomeric golgi complex 1              | 0.19              | 0.18                   | 1.04                          | 1.29e-03    |
| COPB1     | COPI coat complex subunit beta 1                     | 0.02              | 0.02                   | 0.24                          | 1.79e-05    |
| CREB3L1   | cAMP responsive element binding protein 3 like 1     | 0.07              | 0.07                   | 0.37                          | 1.63e-02    |
| CYR1*     | cryptochrome circadian regulator 1                   | 0.07              | 0.06                   | 0.54                          | 9.80e-03    |
| CRYBA1    | crystallin beta A1                                   | 0.05              | 0.04                   | 0.59                          | 3.22e-02    |
| CUTC      | cutC copper transporter                               | 0.11              | 0.1                    | 1.23                          | 8.48e-03    |
| CYP2R1*   | cytochrome P450 family 2 subfamily R member 1        | 0.19              | 0.18                   | 1.99                          | 8.29e-03    |
| DAZAP2    | DAZ associated protein 2                             | 0.01              | 0                      | 0.17                          | 2.98e-02    |
| DCLK3     | doublecortin like kinase 3                           | 0.19              | 0.18                   | 0.63                          | 3.88e-02    |
| DANN2*    | DANN replication helicase/nuclease 2                 | 0.15              | 0.15                   | 0.42                          | 4.21e-02    |
| DOLK      | dolichol kinase                                      | 0.11              | 0.09                   | 0.47                          | 3.69e-03    |
| EHD3      | EH domain containing 3                               | 0.05              | 0.03                   | 2.69                          | 1.93e-11    |
| EHHADH*   | enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase | 0.28          | 0.26                   | 0.79                          | 1.63e-02    |
| EIF3M     | eukaryotic translation initiation factor 3 subunit M  | 0.01              | 0.01                   | 0.73                          | 7.46e-04    |
| EIF4A1*   | eukaryotic translation initiation factor 4A1         | 0              | 0                      | 0.09                          | 5.77e-03    |
| EXOSC2    | exosome component 2                                  | 0.06              | 0.05                   | 1.17                          | 4.14e-03    |
| FAN1      | FANCD2 and FANCI associated nuclease 1               | 0.4               | 0.39                   | 3.06                          | 1.13e-02    |
| FRMD8     | FERM domain containing 8                             | 0.1               | 0.09                   | 0.39                          | 3.09e-02    |
| FXR2      | FMR1 autosomal homolog 2                              | 0.03              | 0.02                   | 0.27                          | 2.65e-02    |
| GABRD*    | gamma-aminobutyric acid type A receptor subunit delta | 0.03              | 0.03                   | 0.26                          | 1.90e-02    |
| GANAB     | glucosidase II alpha subunit                         | 0.11              | 0.1                    | 0.64                          | 2.10e-03    |
| GAS8*     | growth arrest specific 8                             | 0.03              | 0.03                   | 2.16                          | 7.46e-05    |
| GINS3     | GINS complex subunit 3                               | 0.08              | 0.07                   | 0.94                          | 1.78e-02    |
| GJA3      | gap junction protein alpha 3                          | 0.12              | 0.11                   | 0.99                          | 4.44e-02    |
| Gene name | Gene description                                               | $\omega$ model0 | $\omega$ background | $\omega$ foreground branch | $p$-value  |
|-----------|----------------------------------------------------------------|----------------|----------------------|-----------------------------|------------|
| GMEB2     | glucocorticoid modulatory element binding protein 2            | 0.03           | 0.03                 | 0.19                        | 3.27e-02   |
| GMPPA     | GDP-mannose pyrophosphorylase A                                | 0.04           | 0.03                 | 0.37                        | 3.31e-02   |
| GOLGA5    | golgin A5                                                       | 0.15           | 0.15                 | 0.67                        | 1.48e-02   |
| GOLGA7    | golgin A7                                                      | 0.03           | 0.03                 | 4.07                        | 3.66e-02   |
| GOT2      | glutamic-oxaloacetic transaminase 2                           | 0.07           | 0.06                 | 0.76                        | 4.24e-02   |
| GPR183    | G protein-coupled receptor 183                                | 0.11           | 0.1                  | 1.66                        | 2.21e-02   |
| GPR31     | G protein-coupled receptor 31                                  | 0.19           | 0.18                 | 4.51                        | 5.49e-03   |
| GPR39*    | G protein-coupled receptor 39                                  | 0.12           | 0.12                 | 1.24                        | 1.51e-02   |
| GPR75*    | G protein-coupled receptor 75                                  | 0.16           | 0.15                 | 1.24                        | 3.42e-03   |
| H1-5      | H1.5 linker histone, cluster member                           | 0.05           | 0.04                 | 0.42                        | 4.09e-02   |
| HCFC2a    | host cell factor C2                                            | 0.15           | 0.15                 | 0.87                        | 2.55e-02   |
| HEXIM2a   | HEXIM P-TEFb complex subunit 2                                | 0.14           | 0.13                 | 0.66                        | 3.81e-02   |
| HHIPL2    | HHIP like 2                                                    | 0.19           | 0.18                 | 0.7                         | 2.16e-02   |
| HSPA9b    | heat shock protein family A (Hsp70) member 9                   | 0.09           | 0.08                 | 1.04                        | 2.69e-02   |
| ING1      | inhibitor of growth family member 1                           | 0.03           | 0.03                 | 1.06                        | 3.74e-04   |
| INTS11    | integrator complex subunit 11                                  | 0.01           | 0.01                 | 0.26                        | 1.76e-03   |
| ITGA3b    | integrin subunit alpha 3                                       | 0.13           | 0.12                 | 0.59                        | 4.63e-03   |
| JVD       | isovaleryl-CoA dehydrogenase                                   | 0.1            | 0.09                 | 3.44                        | 1.08e-02   |
| KANSL2a   | KAT8 regulatory NSL complex subunit 2                          | 0.06           | 0.06                 | 0.72                        | 4.29e-02   |
| KATNBL1   | katanin regulatory subunit B1 like 1                           | 0.15           | 0.15                 | 1.1                         | 4.37e-02   |
| KCNF11,3  | potassium voltage-gated channel modifier subfamily F member 1 | 0.02           | 0.02                 | 0.16                        | 1.68e-02   |
| KIAA1324L1| KIAA1324 like                                                  | 0.1            | 0.1                  | 0.79                        | 1.98e-02   |
| KPNB2     | karyopherin subunit alpha 2                                    | 0.18           | 0.15                 | 1.48                        | 3.51e-03   |
| KRT14a    | keratin 14                                                     | 0.12           | 0.11                 | 0.83                        | 2.62e-02   |
| KRT18a    | keratin 18                                                     | 0.1            | 0.1                  | 0.86                        | 4.55e-02   |
| KRT32a    | keratin 32                                                     | 0.2            | 0.18                 | 1.08                        | 9.31e-04   |
| Gene name        | Gene description                                      | $\omega$ model0 | $\omega$ background | $\omega$ foreground branch | $p$-value   |
|------------------|-------------------------------------------------------|----------------|---------------------|-----------------------------|-------------|
| **LENG1**        | leukocyte receptor cluster member 1                   | 0.17           | 0.16                | 1.12                        | 3.00e-02    |
| **LIXI**         | limb and CNS expressed 1                              | 0.05           | 0.04                | 0.68                        | 2.48e-02    |
| **LMOD1**        | leiomodin 1                                            | 0.16           | 0.15                | 1.49                        | 3.39e-02    |
| **LPO**          | lactoperoxidase                                        | 0.23           | 0.22                | 1.62                        | 3.10e-04    |
| **LRRC34**       | leucine rich repeat containing 34                     | 0.17           | 0.16                | 1.3                         | 3.62e-04    |
| **LRRC8D**       | leucine rich repeat containing 8 VRAC subunit D       | 0.03           | 0.03                | 0.24                        | 2.90e-03    |
| **MED15**        | mediator complex subunit 15                           | 0.12           | 0.11                | 0.43                        | 2.13e-02    |
| **MEIOB**        | meiosis specific with OB-fold                         | 0.21           | 0.2                 | 1.2                         | 3.68e-02    |
| **MFSD13A**      | major facilitator superfamily domain containing 13A   | 0.12           | 0.11                | 0.7                         | 3.31e-02    |
| **MIOX**         | myo-inositol oxygenase                                | 0.08           | 0.07                | 1.16                        | 4.86e-03    |
| **MKKS**         | McKusick-Kaufman syndrome                             | 0.34           | 0.33                | 1.45                        | 4.37e-02    |
| **MMAB**         | metabolism of cobalamin associated B                  | 0.18           | 0.17                | 2.34                        | 2.71e-02    |
| **MRGPRF**       | MAS related GPR family member F                       | 0.08           | 0.07                | 0.62                        | 2.90e-02    |
| **MRGPRG**       | MAS related GPR family member G                       | 0.14           | 0.12                | 1.03                        | 7.21e-03    |
| **MRT04**        | MRT4 homolog, ribosome maturation factor              | 0.08           | 0.07                | 0.97                        | 1.80e-02    |
| **NAPIL3**       | nucleosome assembly protein 1 like 3                  | 0.2            | 0.19                | 2.38                        | 2.30e-03    |
| **NDEL1**        | nudE neurodevelopment protein 1 like 1               | 0.05           | 0.04                | 0.38                        | 4.58e-02    |
| **NEUROD4**      | neuronal differentiation 4                            | 0.14           | 0.13                | 1.13                        | 4.04e-02    |
| **NIPAL1**       | NIPA like domain containing 1                         | 0.19           | 0.19                | 1.8                         | 2.12e-02    |
| **NMD3**         | NMD3 ribosome export adaptor                          | 0.1            | 0.09                | 1.05                        | 2.30e-02    |
| **NME9**         | NME/NM23 family member 9                              | 0.31           | 0.29                | 1.48                        | 2.86e-02    |
| **NMT2**         | N-myristoyltransferase 2                               | 0.07           | 0.08                | 0.14                        | 4.04e-02    |
| **NOC3L**        | NOC3 like DNA replication regulator                   | 0.12           | 0.12                | 0.89                        | 1.03e-02    |
| **NSA2**         | NSA2 ribosome biogenesis factor                       | 0.06           | 0.05                | 0.94                        | 1.25e-03    |
| **NSUN5**        | NOP2/Sun RNA methyltransferase 5                      | 0.16           | 0.15                | 0.9                         | 3.55e-02    |
| **OR13C9**       | olfactory receptor family 13 subfamily C member 9     | 0.26           | 0.22                | 1.71                        | 4.65e-03    |
| Gene name | Gene description                                                                 | $\omega$ model0 | $\omega$ background | $\omega$ foreground branch | p-value      |
|-----------|----------------------------------------------------------------------------------|-----------------|---------------------|-----------------------------|-------------|
| OR51F1    | olfactory receptor family 51 subfamily F member 1 (gene/pseudogene)              | 0.23            | 0.21                | 1.27                        | 4.35e-03    |
| OR8B3     | olfactory receptor family 8 subfamily B member 3                                 | 0.21            | 0.18                | 1.13                        | 3.06e-03    |
| OSBPL3    | oxysterol binding protein like 3                                                 | 0.15            | 0.14                | 0.68                        | 2.35e-02    |
| OSTC      | oligosaccharyltransferase complex non-catalytic subunit                          | 0.2             | 0.17                | 1.56                        | 4.60e-02    |
| OTUD3     | OTU deubiquitinase 3                                                             | 0.26            | 0.23                | 2.76                        | 4.35e-03    |
| OVGPI     | oviductal glycoprotein 1                                                         | 0.38            | 0.36                | 1.57                        | 4.19e-02    |
| PANX3     | pannexin 3                                                                       | 0.17            | 0.16                | 0.94                        | 1.80e-02    |
| PCOLCE2   | procollagen C-endopeptidase enhancer 2                                           | 0.19            | 0.18                | 1.01                        | 3.37e-02    |
| PDILT     | protein disulfide isomerase like, testis expressed                               | 0.35            | 0.34                | 1.22                        | 4.83e-02    |
| PERP      | p53 apoptosis effector related to PMP22                                          | 0.08            | 0.07                | 0.96                        | 8.52e-03    |
| PHKG2     | phosphorylase kinase catalytic subunit gamma 2                                   | 0.1             | 0.09                | 1.57                        | 3.51e-03    |
| PLA2G4F   | phospholipase A2 group IVF                                                        | 0.24            | 0.23                | 0.68                        | 3.59e-02    |
| PLEKHA1   | pleckstrin homology domain containing A1                                         | 0.09            | 0.08                | 0.57                        | 4.34e-02    |
| PLEKHG6   | pleckstrin homology and RhoGEF domain containing G6                             | 0.24            | 0.23                | 0.81                        | 3.05e-03    |
| POLE3     | DNA polymerase epsilon 3, accessory subunit                                      | 0.03            | 0.02                | 0.33                        | 2.13e-02    |
| PPP2R2D   | protein phosphatase 2 regulatory subunit Bdelta                                   | 0.04            | 0.03                | 0.32                        | 3.90e-02    |
| PRKAG1    | protein kinase AMP-activated non-catalytic subunit gamma 1                       | 0.02            | 0.01                | 0.43                        | 4.89e-03    |
| PRPF38A   | pre-mRNA processing factor 38A                                                   | 0.1             | 0.08                | 0.73                        | 1.53e-04    |
| PSMB4     | proteasome 20S subunit beta 4                                                     | 0.09            | 0.08                | 1.13                        | 1.42e-02    |
| PSMD5     | proteasome 26S subunit, non-ATPase 5                                             | 0.14            | 0.14                | 1.29                        | 3.49e-02    |
| PTPN2*    | protein tyrosine phosphatase non-receptor type 2                                 | 0.17            | 0.16                | 1.41                        | 2.86e-02    |
| PTX4      | pentraxin 4                                                                      | 0.28            | 0.27                | 1.02                        | 3.74e-02    |
| RAD54B    | RAD54 homolog B                                                                  | 0.24            | 0.23                | 0.94                        | 2.62e-02    |
| RAD54L1   | RAD54 like                                                                       | 0.07            | 0.06                | 1.92                        | 1.85e-04    |
| RASA2*    | RAS p21 protein activator 2                                                       | 0.15            | 0.14                | 0.88                        | 2.14e-02    |
| RASD1*    | ras related dexamethasone induced 1                                              | 0.01            | 0.01                | 0.31                        | 1.09e-02    |
| Gene name     | Gene description                                      | ω model0 | ω background | ω foreground branch | p-value   |
|---------------|-------------------------------------------------------|----------|--------------|---------------------|-----------|
| RASGEF1C     | RasGEF domain family member 1C                       | 0.05     | 0.05         | 0.4                 | 2.83e-02  |
| RDH5         | retinol dehydrogenase 5                              | 0.16     | 0.15         | 0.59                | 4.89e-02  |
| RFX6         | regulatory factor X6                                  | 0.12     | 0.12         | 1.12                | 3.29e-02  |
| RIOK1        | RIO kinase 1                                          | 0.26     | 0.25         | 1.8                 | 3.05e-03  |
| RPS13        | ribosomal protein S13                                 | 0.11     | 0.09         | 1.16                | 3.55e-03  |
| RPS3A        | ribosomal protein S3A                                 | 0.11     | 0.1          | 1.09                | 4.69e-02  |
| RRP12        | ribosomal RNA processing 12 homolog                  | 0.09     | 0.09         | 0.29                | 3.64e-02  |
| RSAD1        | radical S-adenosyl methionine domain containing 1     | 0.13     | 0.12         | 1.23                | 1.76e-02  |
| RUNDC1       | RUN domain containing 1                               | 0.12     | 0.11         | 0.78                | 2.49e-02  |
| SART3        | spliceosome associated factor 3, U4/U6 recycling protein | 0.08   | 0.08         | 0.45                | 1.64e-02  |
| SCYL1        | SCY1 like pseudokinase 1                             | 0.11     | 0.11         | 0.36                | 2.22e-02  |
| SEC14L3      | SEC14 like lipid binding 3                            | 0.07     | 0.06         | 0.78                | 8.78e-06  |
| SERINC1      | serine incorporator 1                                 | 0.06     | 0.06         | 1.33                | 2.79e-03  |
| SERINC3      | serine incorporator 3                                 | 0.3      | 0.29         | 1.28                | 4.19e-02  |
| SH2D5        | SH2 domain containing 5                              | 0.11     | 0.11         | 14.02               | 9.43e-03  |
| SLC16A14     | solute carrier family 16 member 14                    | 0.14     | 0.13         | 0.57                | 4.52e-02  |
| SLC22A7      | solute carrier family 22 member 7                     | 0.27     | 0.26         | 0.78                | 2.93e-02  |
| SLC24A3      | solute carrier family 24 member 3                     | 0.03     | 0.01         | 0.48                | 1.25e-03  |
| SLC2A2       | solute carrier family 2 member 2                       | 0.31     | 0.29         | 0.68                | 9.43e-03  |
| SLC30A2      | solute carrier family 30 member 2                     | 0.15     | 0.14         | 0.54                | 4.24e-02  |
| SLITRK6      | SLIT and NTRK like family member 6                    | 0.11     | 0.11         | 0.73                | 2.37e-02  |
| SMG8         | SMG8 nonsense mediated mRNA decay factor              | 0.06     | 0.06         | 0.32                | 4.55e-02  |
| SMPDL3A      | sphingomyelin phosphodiesterase acid like 3A          | 0.2      | 0.19         | 1.48                | 3.88e-02  |
| SMPDL3B      | sphingomyelin phosphodiesterase acid like 3B          | 0.23     | 0.23         | 22.39               | 4.26e-02  |
| SMYD4        | SET and MYND domain containing 4                      | 0.36     | 0.35         | 1.59                | 2.85e-02  |
| Gene name | Gene description                        | $\omega_{model0}$ | $\omega_{background}$ | $\omega_{foreground\ branch}$ | $p$-value  |
|-----------|----------------------------------------|-------------------|------------------------|-------------------------------|------------|
| SNX15     | sorting nexin 15                       | 0.12              | 0.11                   | 1.34                          | 1.47e-02   |
| SRM        | spermidine synthase                    | 0.1               | 0.08                   | 3.86                          | 1.96e-04   |
| SRP68      | signal recognition particle 68         | 0.05              | 0.04                   | 0.35                          | 2.04e-02   |
| SSTR1      | somatostatin receptor 1                | 0.01              | 0                      | 0.18                          | 2.42e-03   |
| STMND1     | stathmin domain containing 1           | 0.38              | 0.36                   | 1.53                          | 4.66e-03   |
| STRN4      | striatin 4                             | 0.04              | 0.04                   | 0.19                          | 2.88e-02   |
| SYMPK      | symplekin                              | 0.03              | 0.03                   | 0.14                          | 9.86e-03   |
| SYT12      | synaptotagmin 12                       | 0.05              | 0.05                   | 0.55                          | 8.96e-03   |
| TAAR5      | trace amine associated receptor 5      | 0.15              | 0.13                   | 2.12                          | 1.05e-03   |
| TAF6       | TATA-box binding protein associated    | 0.02              | 0.02                   | 0.22                          | 2.59e-02   |
| TCTE1      | t-complex-associated-testis-expressed  | 0.14              | 0.13                   | 1.27                          | 2.03e-02   |
| TGS1       | trimethylguanosine synthase 1          | 0.37              | 0.36                   | 1.53                          | 1.12e-02   |
| THOC3      | THO complex 3                          | 0.01              | 0                      | 0.22                          | 1.43e-03   |
| TINAGL1    | tubulointerstitial nephritis antigen   | 0.1               | 0.09                   | 0.54                          | 3.83e-02   |
| TM6SF1     | transmembrane 6 superfamily member 1   | 0.11              | 0.11                   | 0.8                           | 1.91e-02   |
| TMPRSS2    | transmembrane serine protease 2        | 0.18              | 0.17                   | 0.77                          | 1.88e-02   |
| TNFAIP1    | TNF alpha induced protein 1            | 0.03              | 0.03                   | 0.5                           | 2.32e-02   |
| TRMT5      | tRNA methyltransferase 5               | 0.28              | 0.27                   | 1.76                          | 4.63e-02   |
| TSH2       | teashirt zinc finger homeobox 2        | 0.08              | 0.07                   | 0.38                          | 3.42e-03   |
| TTC38      | tetratricopeptide repeat domain 38     | 0.15              | 0.14                   | 0.84                          | 2.93e-02   |
| TWF1       | twinfilin actin binding protein 1      | 0.06              | 0.05                   | 0.98                          | 6.49e-03   |
| TWISTNB    | TWIST neighbor                         | 0.24              | 0.23                   | 1.21                          | 3.02e-02   |
| TYRP1      | tyrosinase related protein 1           | 0.19              | 0.19                   | 0.95                          | 4.42e-02   |
| USP14      | ubiquitin specific peptidase 14        | 0.05              | 0.05                   | 1.03                          | 9.22e-03   |
| USP30      | ubiquitin specific peptidase 30        | 0.1               | 0.1                    | 0.48                          | 2.34e-02   |
| USP33      | ubiquitin specific peptidase 33        | 0.07              | 0.07                   | 0.25                          | 4.37e-02   |
| Gene name | Gene description | $\omega_{\text{model0}}$ | $\omega_{\text{background}}$ | $\omega_{\text{foreground branch}}$ | $p$-value |
|-----------|------------------|-------------------------|--------------------------|--------------------------------|-----------|
| USP5$^1$ | ubiquitin specific peptidase 5 | 0.03 | 0.03 | 0.3 | 1.69e-02 |
| UTP15$^4$ | UTP15 small subunit processome component | 0.12 | 0.12 | 1.09 | 4.00e-02 |
| VPS16$^2$,4 | VPS16 core subunit of CORVET and HOPS complexes | 0.05 | 0.05 | 0.44 | 7.50e-03 |
| VPS29 | VPS29 retromer complex component | 0.02 | 0.01 | 1.03 | 1.59e-03 |
| WRNIP1$^1$ | WRN helicase interacting protein 1 | 0.03 | 0.03 | 0.82 | 1.01e-02 |
| XPNPEP1 | X-prolyl aminopeptidase 1 | 0.08 | 0.07 | 0.45 | 4.78e-03 |
| XRCC3$^1$ | X-ray repair cross complementing 3 | 0.15 | 0.15 | 2.4 | 7.79e-03 |
| YTHDF1$^1$ | YTH N6-methyladenosine RNA binding protein 1 | 0.06 | 0.06 | 0.22 | 4.26e-02 |
| ZNF395$^*$ | zinc finger protein 395 | 0.16 | 0.15 | 0.76 | 3.88e-02 |
| ZNF641$^2$ | zinc finger protein 641 | 0.16 | 0.16 | 1.22 | 1.83e-02 |

Supplementary Table S9.

Genes with signs of slower evolution on the Steller’s sea cow branch. $P$-value calculated with a chi-square test based on the likelihood ratio test between model 0 and model 2 in CODEML (49) (1 degree of freedom). More slowly evolving genes on the Steller’s sea cow lineage associated with energy homeostasis regulation and body weight are marked with * (literature search via Pubmed, OMIM and Mouse Genome Informatics (http://www.informatics.jax.org)).

1faster evolving in cetaceans; 2more slowly evolving in cetaceans; 3faster evolving in pinnipeds; 4more slowly evolving in pinnipeds.
| Gene name   | Gene description                          | $\omega_{\text{model0}}$ | $\omega_{\text{background}}$ | $\omega_{\text{foreground branch}}$ | $p$-value   |
|-------------|-------------------------------------------|---------------------------|--------------------------------|--------------------------------------|-------------|
| CD79B       | CD79b molecule                            | 0.28                      | 0.29                          | 0                                    | 3.90e-02    |
| CDH3*       | cadherin 3                                | 0.15                      | 0.16                          | 0                                    | 1.37e-02    |
| CFAP157     | cilia and flagella associated protein 157 | 0.21                      | 0.21                          | 0                                    | 2.45e-02    |
| CHGB        | chromogranin B                            | 0.36                      | 0.37                          | 0                                    | 4.29e-02    |
| CSNKA2IP    | casein kinase 2 subunit alpha' interacting protein | 0.49                      | 0.51                          | 0                                    | 7.41e-03    |
| DBF4        | DBF4 zinc finger                          | 0.33                      | 0.33                          | 0                                    | 3.70e-02    |
| DPP9        | dipeptidyl peptidase 9                    | 0.05                      | 0.05                          | 0                                    | 4.19e-02    |
| FASTKD1     | FAST kinase domains 1                     | 0.42                      | 0.43                          | 0.06                                 | 2.96e-02    |
| FASTKD2     | FAST kinase domains 2                     | 0.48                      | 0.5                           | 0.1                                  | 6.74e-03    |
| GFRA3       | GDNF family receptor alpha 3              | 0.22                      | 0.23                          | 0                                    | 1.26e-02    |
| GP1BA*      | glycoprotein Ib platelet subunit alpha     | 0.51                      | 0.52                          | 0.14                                 | 4.97e-02    |
| IARS2       | isoleucyl-tRNA synthetase 2, mitochondrial | 0.14                      | 0.15                          | 0                                    | 3.97e-02    |
| IL4R        | interleukin 4 receptor                    | 0.42                      | 0.43                          | 0.11                                 | 3.05e-02    |
| IL5*        | interleukin 5                             | 0.59                      | 0.63                          | 0                                    | 2.58e-02    |
| IRF5        | interferon regulatory factor 5            | 0.1                       | 0.1                           | 0                                    | 1.54e-02    |
| LRIF1       | ligand dependent nuclear receptor interacting factor 1 | 0.64                      | 0.65                          | 0                                    | 3.59e-02    |
| LURAP1      | leucine rich adaptor protein 1            | 0.17                      | 0.18                          | 0                                    | 2.11e-02    |
| MAEA        | macrophage erythroblast attacher          | 0.01                      | 0.01                          | 0                                    | 1.69e-03    |
| MUTYH*      | mutY DANN glycosylase                     | 0.3                       | 0.3                           | 0                                    | 6.10e-03    |
| NEU3        | neuraminidase 3                           | 0.34                      | 0.35                          | 0                                    | 1.29e-02    |
| NHLRC3      | NHL repeat containing 3                   | 0.2                       | 0.21                          | 0                                    | 3.81e-02    |
| NPR1        | natriuretic peptide receptor 1            | 0.06                      | 0.06                          | 0                                    | 4.31e-02    |
| PEX11A*     | peroxisomal biogenesis factor 11 alpha    | 0.31                      | 0.32                          | 0                                    | 3.33e-02    |
| PLA2G2A*    | phospholipase A2 group IIA                | 0.49                      | 0.54                          | 0                                    | 2.38e-02    |
| PXMP4*      | peroxisomal membrane protein 4            | 0.15                      | 0.16                          | 0                                    | 2.07e-04    |
| SLFN14*     | schlafen family member 14                 | 0.38                      | 0.38                          | 0                                    | 4.14e-02    |
Pathway enrichment analysis

Twenty out of 238 genes that we identified to have significantly different evolutionary rates in Steller’s sea cow have also been shown in other studies to modulate body weight and energy homeostasis (marked with * in Supplementary Tables S8 and S9). For example, BCO1 (faster evolving in Steller’s sea cow) is a β-carotenoid-15,15’-oxygenase that generates vitamin A from β-carotene. Interestingly, BCO1-deficient mice were more susceptible to high fat diet-induced impairments in fatty acid metabolism, an indirect effect probably related to hypercarotenemia and vitamin A deficiency (64). The activity of adenosine monophosphate deaminase 2 (AMPD2) (more slowly evolving in Steller’s sea cow) positively correlates with hepatic fat synthesis and fat oxidation rates (65). Members of the family of bone morphogenetic proteins (BMP) are important regulators of adipogenesis. Genetic variants of the BMP receptor 1A gene (BMPR1A) (faster evolving in Steller’s sea cow) and its expression levels in adipose tissue have been associated with overweight and obesity (66). GPR39 (faster evolving in Steller’s sea cow, cetaceans, and pinnipeds) is a constitutively active G protein-coupled receptor and variants have been associated with obesity. Gpr39 deficiency in mice causes increased fat accumulation on a high-fat diet, conceivably due to decreased energy expenditure and adipocyte lipolytic activity and altered diet-induced thermogenesis (67, 68). Recently, GPR75 variants have been found to protect from obesity and mice deficient for GPR75 show resistance to weight gain when fed a high-caloric diet (69). The significantly high number of genes associated with energy homeostasis regulation and body weight (hypergeometric test p-value = 0.006) suggests that metabolic pathways involved in the switch of the energetic balance towards fat accumulation contributed to evolutionary adaptations of Steller’s sea cow.

To further explore which functions may have experienced different evolutionary pressures along the lineage to Steller’s sea cow, we tested GO enrichment using the FUNC package (55). We ran a hypergeometric test in which the significantly differently evolving genes were the set of interest. We considered the remaining genes from the 4,877 1:1 orthologs as the background genes.
Several categories with an uncorrected \( p \)-value < 0.05 fit well with the Steller’s sea cow phenotype: e.g. long-chain fatty acid-CoA ligase activity – GO:0004467, low-density lipoprotein particle remodeling – GO:0034374, negative regulation of epidermis development – GO:0045683, regulation of melanin biosynthetic process – GO:0048021 or negative regulation of hair follicle maturation GO:0048817 (Supplementary Table S10).

It is necessary for mammals to maintain constant body temperature in cold environments. In addition to muscle shivering, non-shivering thermogenesis plays a major role in thermal homeostasis. There are two major forms of non-shivering thermogenesis (NST): uncoupling protein 1 (UCP1)-dependent and UCP1-independent. The UCP1-dependent thermogenesis relies on the ability of the brown adipose tissue (BAT) to produce heat based on uncoupling the electron transport from mitochondrial ATP synthesis. UCP1 is absent in several mammals, such as pigs, Steller’s sea cow, mammoths, and elephants (25); these species must generate heat (if the environment does not provide sufficient temperature) by UCP1-independent mechanisms. There are several thermogenic futile cycles “wasting” ATP by hydrolysis to generate heat, among them the lipolysis/re-esterification cycle, which uses ATP to synthesize acylglycerol (27, 28). Formation of acylglycerols requires CoA-activated fatty acids, which is the ATP-consuming process. \( ACSF3 \) and \( ACSL5 \), both with significantly different evolutionary rates in Steller’s sea cow, encode for acyl-CoA synthetases that activate long chain fatty acids (72). It is therefore likely that changes in their function influence this thermogenic futile cycle. Alternatively, fatty acids released from the massive fat depots of the Steller’s sea cow could have been activated by both acyl-CoA synthetases and introduced into the \( \beta \)-oxidation to supply ATP, e.g. under fasting conditions. The gene encoding for enoyl-CoA-hydratase:3-hydroxyacyl-CoA dehydrogenase (encoded by \( EHHADH \)) is also among the faster evolving genes in Steller’s sea cow (Supplementary Table S8), supporting this latter point. This enzyme is involved in peroxisomal oxidation of fatty acids. Similarly, the faster evolving isovaleryl-CoA dehydrogenase (encoded by \( IVD \), Supplementary Table S8) is a short and medium chain acyl-CoA dehydrogenase also participating in acyl component degradation.

Thus, it seems that differentially evolving genes on the Steller’s sea cow lineage are involved in lipid-related metabolic pathways, which could explain their thick blubber, up to 10 cm in some areas of the body (4).

Supplementary Table S10.

| Node name                                      | Node ID     | No. of genes in node | No. of significant genes in node | Uncorrected \( p \)-value |
|------------------------------------------------|-------------|----------------------|----------------------------------|--------------------------|
| mismatch base pair DNA N-glycosylase activity | GO:0000700  | 4                    | 1                                | 0.03                     |
| purine-specific mismatch base pair DNA N-     | GO:0000701  | 2                    | 1                                | 0.02                     |
| glycosylase activity                           |             |                      |                                  |                          |
| Node name                                                                 | Node ID    | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|--------------------------------------------------------------------------|------------|----------------------|----------------------------------|---------------------|
| oxidized base lesion DNA N-glycosylase activity                          | GO:0000702 | 1                    | 1                                | 0.01                |
| peptide receptor activity                                                | GO:0001653 | 43                   | 2                                | 0.05                |
| aminoacyl-tRNA editing activity                                          | GO:0002161 | 4                    | 1                                | 0.03                |
| DNA-(apurinic or apyrimidinic site) lyase activity                       | GO:0003906 | 3                    | 1                                | 0.02                |
| acyl-CoA ligase activity                                                 | GO:0003996 | 5                    | 1                                | 0.04                |
| exo-alpha-sialidase activity                                             | GO:0004308 | 1                    | 1                                | 0.01                |
| guanylate cyclase activity                                               | GO:0004383 | 4                    | 1                                | 0.03                |
| long-chain fatty acid-CoA ligase activity                                | GO:0004467 | 5                    | 1                                | 0.04                |
| endonuclease activity                                                    | GO:0004519 | 41                   | 2                                | 0.05                |
| phospholipase activity                                                   | GO:0004620 | 43                   | 2                                | 0.05                |
| sphingomyelin phosphodiesterase activity                                 | GO:0004767 | 5                    | 1                                | 0.04                |
| transposase activity                                                     | GO:0004803 | 1                    | 1                                | 0.01                |
| isoleucine-tRNA ligase activity                                          | GO:0004822 | 1                    | 1                                | 0.01                |
| signal transducer activity                                               | GO:0004871 | 342                  | 7                                | 0.02                |
| transmembrane signaling receptor activity                                | GO:0004888 | 247                  | 6                                | 0.02                |
| cytokine receptor activity                                               | GO:0004896 | 37                   | 2                                | 0.04                |
| interleukin-4 receptor activity                                          | GO:0004913 | 1                    | 1                                | 0.01                |
| laminin receptor activity                                                | GO:0005055 | 1                    | 1                                | 0.01                |
| interleukin-5 receptor binding                                           | GO:0005137 | 1                    | 1                                | 0.01                |
| sodium:potassium-exchanging ATPase activity                              | GO:0005391 | 4                    | 2                                | 0                  |
| axon guidance receptor activity                                          | GO:0008046 | 2                    | 1                                | 0.02                |
| G protein-coupled peptide receptor activity                              | GO:0008528 | 41                   | 2                                | 0.05                |
| oxidized purine nucleobase lesion DNA N-glycosylase activity             | GO:0008534 | 1                    | 1                                | 0.01                |
| potassium-transporting ATPase activity                                   | GO:0008556 | 4                    | 2                                | 0                  |
| hydrogen:potassium-exchanging ATPase activity                            | GO:0008900 | 1                    | 1                                | 0.01                |
| cyclase activity                                                         | GO:0009975 | 6                    | 1                                | 0.05                |
| glucuronosyltransferase activity                                         | GO:0015020 | 2                    | 1                                | 0.02                |
| thrombin-activated receptor activity                                     | GO:0015057 | 2                    | 1                                | 0.02                |
| potassium ion transmembrane transporter activity                         | GO:0015079 | 38                   | 2                                | 0.04                |
| primary active transmembrane transporter activity                        | GO:0015399 | 32                   | 2                                | 0.03                |
| P-P-bond-hydrolysis-driven transmembrane transporter activity            | GO:0015405 | 32                   | 2                                | 0.03                |
| ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism | GO:0015662 | 12                   | 2                                | 0                  |
| glial cell-derived neurotrophic factor receptor activity                 | GO:0016167 | 1                    | 1                                | 0.01                |
| CoA-ligase activity                                                      | GO:0016405 | 5                    | 1                                | 0.04                |
| oxidoreductase activity, acting on a sulfur group of donors, NAD(P) as acceptor | GO:0016668 | 5                    | 1                                | 0.04                |
| Node name                                                                 | Node ID    | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|--------------------------------------------------------------------------|------------|----------------------|----------------------------------|---------------------|
| hydrolase activity, acting on glycosyl bonds                             | GO:0016798 | 46                   | 3                                | 0.01                |
| hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances | GO:0016820 | 28                   | 2                                | 0.02                |
| phosphorus-oxygen lyase activity                                         | GO:0016849 | 6                    | 1                                | 0.05                |
| natriuretic peptide receptor activity                                   | GO:0016941 | 2                    | 1                                | 0.02                |
| alpha-sialidase activity                                                 | GO:0016997 | 1                    | 1                                | 0.01                |
| DNA N-glycosylase activity                                               | GO:0019104 | 4                    | 1                                | 0.03                |
| kinase activator activity                                                | GO:0019209 | 27                   | 2                                | 0.02                |
| cation-transporting ATPase activity                                       | GO:0021829 | 17                   | 2                                | 0.01                |
| active ion transmembrane transporter activity                            | GO:0022853 | 33                   | 2                                | 0.03                |
| protein kinase activator activity                                       | GO:0030295 | 24                   | 2                                | 0.02                |
| transmembrane receptor protein tyrosine kinase activator activity        | GO:0030297 | 2                    | 1                                | 0.02                |
| oxidized DNA binding                                                    | GO:0032356 | 3                    | 1                                | 0.02                |
| oxidized purine DNA binding                                              | GO:0032357 | 3                    | 1                                | 0.02                |
| mismatch repair complex binding                                          | GO:0032404 | 4                    | 1                                | 0.03                |
| Mutalpha complex binding                                                 | GO:0032407 | 2                    | 1                                | 0.02                |
| 8-oxo-7,8-dihydroguanine DNA N-glycosylase activity                     | GO:0034039 | 1                    | 1                                | 0.01                |
| adenine/guanine mispair binding                                          | GO:0035485 | 1                    | 1                                | 0.01                |
| signaling receptor activity                                              | GO:0038023 | 308                  | 6                                | 0.04                |
| ATPase coupled ion transmembrane transporter activity                    | GO:0042625 | 18                   | 2                                | 0.01                |
| ATPase activity, coupled to transmembrane movement of substances         | GO:0042626 | 28                   | 2                                | 0.02                |
| ATPase activity, coupled to movement of substances                       | GO:0043492 | 28                   | 2                                | 0.02                |
| dynein intermediate chain binding                                        | GO:0045505 | 6                    | 1                                | 0.05                |
| protein-disulfide reductase activity                                     | GO:0047134 | 2                    | 1                                | 0.02                |
| arachidonate-CoA ligase activity                                         | GO:0047676 | 4                    | 1                                | 0.03                |
| exo-alpha-(2->3)-sialidase activity                                     | GO:0052794 | 1                    | 1                                | 0.01                |
| exo-alpha-(2->6)-sialidase activity                                     | GO:0052795 | 1                    | 1                                | 0.01                |
| exo-alpha-(2->8)-sialidase activity                                     | GO:0052796 | 1                    | 1                                | 0.01                |
| acid sphingomyelin phosphodiesterase activity                           | GO:0061750 | 1                    | 1                                | 0.01                |
| transmembrane receptor activity                                          | GO:0096000 | 260                  | 6                                | 0.02                |
| decanoate--CoA ligase activity                                           | GO:0102391 | 4                    | 1                                | 0.03                |
| immunoglobulin production                                               | GO:0002377 | 38                   | 2                                | 0.04                |
| regulation of immunoglobulin production                                 | GO:0002637 | 24                   | 2                                | 0.02                |
| positive regulation of immunoglobulin production                        | GO:0002639 | 17                   | 2                                | 0.01                |
| positive regulation of production of molecular mediator of immune response | GO:0002702 | 32                   | 2                                | 0.03                |
| negative regulation of T-helper 1 type immune response                  | GO:0002826 | 2                    | 1                                | 0.02                |
| Node name                                                                 | Node ID      | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|--------------------------------------------------------------------------|--------------|----------------------|----------------------------------|---------------------|
| positive regulation of type 2 immune response                            | GO:0002830   | 6                    | 1                                | 0.05                |
| uronic acid metabolic process                                            | GO:0006063   | 4                    | 1                                | 0.03                |
| base-excision repair, AP site formation                                  | GO:0006285   | 3                    | 1                                | 0.02                |
| transposition, DNA-mediated                                              | GO:0006313   | 1                    | 1                                | 0.01                |
| isoleucyl-tRNA aminoacylation                                             | GO:0006428   | 1                    | 1                                | 0.01                |
| protein phosphorylation                                                   | GO:0006468   | 575                  | 9                                | 0.04                |
| glycolipid metabolic process                                             | GO:0006664   | 42                   | 2                                | 0.05                |
| ceramide metabolic process                                               | GO:0006672   | 37                   | 2                                | 0.04                |
| sphingomyelin catabolic process                                          | GO:0006685   | 5                    | 1                                | 0.04                |
| glycosphingolipid metabolic process                                      | GO:0006687   | 30                   | 2                                | 0.03                |
| ganglioside catabolic process                                            | GO:0006689   | 1                    | 1                                | 0.01                |
| porphyrin-containing compound catabolic process                          | GO:0006787   | 4                    | 1                                | 0.03                |
| bilirubin conjugation                                                    | GO:0006789   | 1                    | 1                                | 0.01                |
| cellular sodium ion homeostasis                                          | GO:0006883   | 6                    | 2                                | 0                   |
| regulation of pH                                                         | GO:0006885   | 31                   | 2                                | 0.03                |
| protein catabolic process in the vacuole                                 | GO:0007039   | 5                    | 1                                | 0.04                |
| lysosomal lumen acidification                                            | GO:0007042   | 5                    | 1                                | 0.04                |
| receptor guanylyl cyclase signaling pathway                              | GO:0007168   | 4                    | 1                                | 0.03                |
| activation of transmembrane receptor protein tyrosine kinase activity     | GO:0007171   | 3                    | 1                                | 0.02                |
| oligosaccharide catabolic process                                        | GO:0009313   | 4                    | 1                                | 0.03                |
| flavonoid metabolic process                                              | GO:0009812   | 3                    | 1                                | 0.02                |
| potassium ion import                                                    | GO:0010107   | 14                   | 2                                | 0.01                |
| establishment or maintenance of transmembrane electrochemical gradient    | GO:0010248   | 6                    | 2                                | 0                   |
| positive regulation of nuclear cell cycle DNA replication                | GO:0010571   | 4                    | 1                                | 0.03                |
| positive regulation of macrophage derived foam cell differentiation       | GO:0010744   | 4                    | 1                                | 0.03                |
| regulation of cGMP-mediated signaling                                    | GO:0010752   | 6                    | 1                                | 0.05                |
| positive regulation of cGMP-mediated signaling                           | GO:0010753   | 4                    | 1                                | 0.03                |
| positive regulation of sodium ion transport                              | GO:0010765   | 5                    | 1                                | 0.04                |
| positive regulation of keratinocyte proliferation                        | GO:0010838   | 6                    | 1                                | 0.05                |
| negative regulation of angiogenesis                                      | GO:0016525   | 34                   | 2                                | 0.03                |
| peroxisome membrane biogenesis                                           | GO:0016557   | 1                    | 1                                | 0.01                |
| peroxisome fission                                                       | GO:0016559   | 4                    | 1                                | 0.03                |
| biphenyl metabolic process                                               | GO:0018879   | 2                    | 1                                | 0.02                |
| phenol-containing compound metabolic process                             | GO:0018958   | 27                   | 2                                | 0.02                |
| glucuronate metabolic process                                            | GO:0019585   | 4                    | 1                                | 0.03                |
| developmental maturation                                                 | GO:0021700   | 72                   | 3                                | 0.02                |
| termination of signal transduction                                       | GO:0023021   | 2                    | 1                                | 0.02                |
| Node name                                                                 | Node ID   | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|---------------------------------------------------------------------------|-----------|----------------------|----------------------------------|---------------------|
| cellular monovalent inorganic cation homeostasis                          | GO:0030004| 32                   | 3                                | 0                   |
| cellular potassium ion homeostasis                                        | GO:0030007| 5                    | 2                                | 0                   |
| sphingolipid catabolic process                                            | GO:0030149| 14                   | 2                                | 0.01                |
| eosinophil differentiation                                                | GO:0030222| 3                    | 1                                | 0.02                |
| regulation of granulocyte differentiation                                 | GO:0030852| 6                    | 1                                | 0.05                |
| positive regulation of granulocyte differentiation                        | GO:0030854| 5                    | 1                                | 0.04                |
| transposition                                                             | GO:0032196| 5                    | 1                                | 0.04                |
| response to peptidoglycan                                                 | GO:0032494| 4                    | 1                                | 0.03                |
| response to muramyl dipeptide                                             | GO:0032495| 6                    | 1                                | 0.05                |
| regulation of monophenol monooxygenase activity                           | GO:0032771| 1                    | 1                                | 0.01                |
| positive regulation of monophenol monooxygenase activity                 | GO:0032773| 1                    | 1                                | 0.01                |
| transforming growth factor beta2 production                               | GO:0032906| 1                    | 1                                | 0.01                |
| regulation of transforming growth factor beta2 production                 | GO:0032909| 1                    | 1                                | 0.01                |
| negative regulation of transforming growth factor beta2 production        | GO:0032912| 1                    | 1                                | 0.01                |
| positive regulation of mast cell activation involved in immune response   | GO:0033008| 6                    | 1                                | 0.05                |
| tetrapyrrole catabolic process                                            | GO:0033015| 4                    | 1                                | 0.03                |
| negative regulation of myeloid cell apoptotic process                    | GO:0033033| 5                    | 1                                | 0.04                |
| low-density lipoprotein particle remodeling                               | GO:0034374| 5                    | 1                                | 0.04                |
| sodium ion transmembrane transport                                       | GO:0035725| 31                   | 2                                | 0.03                |
| interleukin-4-mediated signaling pathway                                  | GO:0035771| 3                    | 1                                | 0.02                |
| positive regulation of urine volume                                       | GO:0035810| 6                    | 1                                | 0.05                |
| glial cell-derived neurotrophic factor receptor signaling pathway         | GO:0035860| 2                    | 1                                | 0.02                |
| cellular response to increased oxygen levels                              | GO:0036295| 4                    | 1                                | 0.03                |
| platelet maturation                                                       | GO:0036345| 2                    | 1                                | 0.02                |
| sodium ion export from cell                                               | GO:0036376| 4                    | 2                                | 0                   |
| heme catabolic process                                                    | GO:0042167| 4                    | 1                                | 0.03                |
| xenobiotic catabolic process                                              | GO:0042178| 5                    | 1                                | 0.04                |
| pigment metabolic process                                                 | GO:0042440| 28                   | 2                                | 0.02                |
| negative regulation of hair cycle                                        | GO:0042636| 3                    | 1                                | 0.02                |
| catagen                                                                   | GO:0042637| 1                    | 1                                | 0.01                |
| defense response to protozoan                                             | GO:0042832| 6                    | 1                                | 0.05                |
| regulation of vascular permeability                                       | GO:0043114| 6                    | 1                                | 0.05                |
| positive regulation of mast cell degranulation                            | GO:0043306| 6                    | 1                                | 0.05                |
| enucleate erythrocyte differentiation                                     | GO:0043353| 2                    | 1                                | 0.02                |
| regulation of secondary metabolic process                                 | GO:0043455| 6                    | 1                                | 0.05                |
| positive regulation of insulin-like growth factor receptor signaling pathway| GO:0043568| 5                    | 1                                | 0.04                |
| Node name                                                  | Node ID      | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|------------------------------------------------------------|--------------|----------------------|---------------------------------|---------------------|
| regulation of peroxisome size                             | GO:0044375   | 2                    | 1                               | 0.02                |
| regulation of mitochondrial mRNA stability                 | GO:0044528   | 2                    | 1                               | 0.02                |
| biological phase                                          | GO:0044848   | 3                    | 1                               | 0.02                |
| hair cycle phase                                          | GO:0044851   | 3                    | 1                               | 0.02                |
| depurination                                              | GO:0045007   | 1                    | 1                               | 0.01                |
| T-helper 2 cell differentiation                           | GO:0045064   | 5                    | 1                               | 0.04                |
| regulation of T-helper 1 cell differentiation              | GO:0045625   | 4                    | 1                               | 0.03                |
| negative regulation of T-helper 1 cell differentiation     | GO:0045626   | 1                    | 1                               | 0.01                |
| regulation of T-helper 2 cell differentiation              | GO:0045628   | 4                    | 1                               | 0.03                |
| positive regulation of T-helper 2 cell differentiation     | GO:0045630   | 2                    | 1                               | 0.02                |
| regulation of eosinophil differentiation                  | GO:0045643   | 2                    | 1                               | 0.02                |
| positive regulation of eosinophil differentiation          | GO:0045645   | 2                    | 1                               | 0.02                |
| negative regulation of epidermis development              | GO:0045683   | 4                    | 1                               | 0.03                |
| pigment catabolic process                                 | GO:0046149   | 4                    | 1                               | 0.03                |
| membrane lipid catabolic process                          | GO:0046466   | 17                   | 2                               | 0.01                |
| glycosphingolipid catabolic process                       | GO:0046479   | 5                    | 1                               | 0.04                |
| regulation of melanin biosynthetic process                | GO:0048021   | 5                    | 1                               | 0.04                |
| positive regulation of melanin biosynthetic process       | GO:0048023   | 3                    | 1                               | 0.02                |
| immunoglobulin secretion                                 | GO:0048305   | 5                    | 1                               | 0.04                |
| autonomic nervous system development                      | GO:0048483   | 6                    | 1                               | 0.05                |
| sympathetic nervous system development                    | GO:0048485   | 5                    | 1                               | 0.04                |
| negative regulation of hair follicle maturation            | GO:0048817   | 2                    | 1                               | 0.02                |
| regulation of hair follicle maturation                    | GO:0048819   | 3                    | 1                               | 0.02                |
| hair follicle maturation                                  | GO:0048820   | 6                    | 1                               | 0.05                |
| enucleate erythrocyte development                         | GO:0048822   | 2                    | 1                               | 0.02                |
| negative regulation of T cell activation                  | GO:0050868   | 36                   | 2                               | 0.04                |
| regulation of immunoglobulin secretion                    | GO:0051023   | 5                    | 1                               | 0.04                |
| positive regulation of immunoglobulin secretion           | GO:0051024   | 3                    | 1                               | 0.02                |
| flavone metabolic process                                 | GO:0051552   | 1                    | 1                               | 0.01                |
| regulation of catagen                                      | GO:0051794   | 1                    | 1                               | 0.01                |
| negative regulation of catagen                            | GO:0051796   | 1                    | 1                               | 0.01                |
| regulation of hair follicle development                   | GO:0051797   | 6                    | 1                               | 0.05                |
| negative regulation of hair follicle development           | GO:0051799   | 3                    | 1                               | 0.02                |
| cellular glucuronidation                                  | GO:0052695   | 3                    | 1                               | 0.02                |
| flavonoid glucuronidation                                 | GO:0052696   | 2                    | 1                               | 0.02                |
| xenobiotic glucuronidation                                | GO:0052697   | 2                    | 1                               | 0.02                |
| monovalent inorganic cation homeostasis                   | GO:0055067   | 45                   | 4                               | 0                   |
| potassium ion homeostasis                                | GO:0055075   | 9                    | 2                               | 0                   |
| sodium ion homeostasis                                    | GO:0055078   | 15                   | 3                               | 0                   |
| Node name                                                                 | Node ID    | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|--------------------------------------------------------------------------|------------|----------------------|---------------------------------|----------------------|
| regulation of hair cycle by canonical Wnt signaling pathway              | GO:0060901| 1                    | 1                               | 0.01                 |
| cardiac conduction                                                       | GO:0061337| 34                   | 2                               | 0.03                 |
| mitochondrial ribosome assembly                                          | GO:0061668| 5                    | 1                               | 0.04                 |
| proton-transporting V-type ATPase complex assembly                        | GO:0070070| 6                    | 1                               | 0.05                 |
| vacuolar proton-transporting V-type ATPase complex assembly              | GO:0070072| 5                    | 1                               | 0.04                 |
| thrombin-activated receptor signaling pathway                            | GO:0070493| 3                    | 1                               | 0.02                 |
| organelle assembly                                                       | GO:0070925| 236                  | 5                               | 0.04                 |
| biphenyl catabolic process                                               | GO:0070980| 1                    | 1                               | 0.01                 |
| cellular response to magnesium ion                                       | GO:0071286| 3                    | 1                               | 0.02                 |
| cellular response to manganese ion                                       | GO:0071287| 4                    | 1                               | 0.03                 |
| cellular response to ethanol                                             | GO:0071361| 4                    | 1                               | 0.03                 |
| sodium ion export                                                        | GO:0071436| 4                    | 2                               | 0                   |
| negative regulation of transforming growth factor beta production         | GO:0071635| 3                    | 1                               | 0.02                 |
| podosome assembly                                                        | GO:0071800| 3                    | 1                               | 0.02                 |
| regulation of podosome assembly                                          | GO:0071801| 2                    | 1                               | 0.02                 |
| positive regulation of podosome assembly                                 | GO:0071803| 2                    | 1                               | 0.02                 |
| chemokine secretion                                                     | GO:0090195| 3                    | 1                               | 0.02                 |
| regulation of chemokine secretion                                       | GO:0090196| 3                    | 1                               | 0.02                 |
| positive regulation of chemokine secretion                              | GO:0090197| 3                    | 1                               | 0.02                 |
| ammonium ion metabolic process                                           | GO:0097164| 65                   | 3                               | 0.02                 |
| import into cell                                                         | GO:0098657| 41                   | 2                               | 0.05                 |
| import across plasma membrane                                           | GO:0098739| 29                   | 2                               | 0.02                 |
| regulation of secondary metabolite biosynthetic process                  | GO:1900376| 5                    | 1                               | 0.04                 |
| positive regulation of secondary metabolite biosynthetic process         | GO:1900378| 3                    | 1                               | 0.02                 |
| negative regulation of vasculature development                           | GO:1901343| 37                   | 2                               | 0.04                 |
| regulation of myoblast fusion                                            | GO:1901739| 6                    | 1                               | 0.05                 |
| positive regulation of myoblast fusion                                  | GO:1901741| 6                    | 1                               | 0.05                 |
| positive regulation of sodium ion transmembrane transport                | GO:1902307| 3                    | 1                               | 0.02                 |
| mitochondrial large ribosomal subunit assembly                           | GO:1902775| 2                    | 1                               | 0.02                 |
| regulation of melanosome transport                                      | GO:1902908| 2                    | 1                               | 0.02                 |
| positive regulation of melanosome transport                              | GO:1902910| 1                    | 1                               | 0.01                 |
| negative regulation of leukocyte cell-cell adhesion                      | GO:1903038| 37                   | 2                               | 0.04                 |
| regulation of sodium ion export                                          | GO:1903273| 1                    | 1                               | 0.01                 |
| positive regulation of sodium ion export                                 | GO:1903275| 1                    | 1                               | 0.01                 |
| regulation of sodium ion export from cell                                | GO:1903276| 1                    | 1                               | 0.01                 |
| positive regulation of sodium ion export from cell                       | GO:1903278| 1                    | 1                               | 0.01                 |
| Node name                                      | Node ID       | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|-----------------------------------------------|---------------|----------------------|---------------------------------|---------------------|
| regulation of potassium ion import           | GO:1903286    | 2                    | 1                               | 0.02                |
| positive regulation of potassium ion import  | GO:1903288    | 1                    | 1                               | 0.01                |
| liposaccharide metabolic process              | GO:1903509    | 42                   | 2                               | 0.05                |
| regulation of cardiac conduction              | GO:1903779    | 17                   | 2                               | 0.01                |
| lysosomal protein catabolic process           | GO:1905146    | 4                    | 1                               | 0.03                |
| potassium ion import across plasma membrane  | GO:1990573    | 13                   | 2                               | 0.01                |
| response to odorant                          | GO:1990834    | 2                    | 1                               | 0.02                |
| positive regulation of DNA-dependent DNA replication | GO:2000105  | 6                    | 1                               | 0.05                |
| negative regulation of blood vessel morphogenesis | GO:2000181  | 34                   | 2                               | 0.03                |
| sex chromosome                               | GO:0000803    | 6                    | 1                               | 0.05                |
| X chromosome                                 | GO:0000805    | 4                    | 1                               | 0.03                |
| Barr body                                    | GO:0001740    | 2                    | 1                               | 0.02                |
| extracellular region                         | GO:0005876    | 1106                 | 15                              | 0.03                |
| vacuolar lumen                               | GO:0005775    | 41                   | 2                               | 0.05                |
| peroxisomal membrane                         | GO:0005778    | 22                   | 2                               | 0.01                |
| integral component of peroxisomal membrane   | GO:0005779    | 6                    | 1                               | 0.05                |
| actomyosin contractile ring                  | GO:0005826    | 1                    | 1                               | 0.01                |
| cytoplasmic dynein complex                   | GO:0005868    | 6                    | 1                               | 0.05                |
| plasma membrane                              | GO:0005886    | 1268                 | 17                              | 0.02                |
| integral component of plasma membrane        | GO:0005887    | 428                  | 9                               | 0.01                |
| hydrogen:potassium-exchanging ATPase complex | GO:0005889    | 1                    | 1                               | 0.01                |
| sodium:potassium-exchanging ATPase complex   | GO:0005890    | 3                    | 1                               | 0.03                |
| external side of plasma membrane             | GO:0009897    | 83                   | 4                               | 0                    |
| cell surface                                 | GO:0009986    | 229                  | 5                               | 0.04                |
| microtubule cytoskeleton                     | GO:0015630    | 344                  | 7                               | 0.02                |
| catenin complex                              | GO:0016342    | 5                    | 1                               | 0.04                |
| nuclear matrix                               | GO:0016363    | 26                   | 2                               | 0.02                |
| vacuolar proton-transporting V-type ATPase complex | GO:0016471  | 5                    | 1                               | 0.04                |
| immunoglobulin complex                       | GO:0019814    | 1                    | 1                               | 0.01                |
| B cell receptor complex                       | GO:0019815    | 1                    | 1                               | 0.01                |
| extrinsic component of membrane              | GO:0019898    | 72                   | 3                               | 0.02                |
| interphase microtubule organizing center      | GO:0031021    | 2                    | 1                               | 0.02                |
| anchored component of membrane               | GO:0031225    | 32                   | 2                               | 0.03                |
| intrinsic component of plasma membrane       | GO:0031226    | 445                  | 9                               | 0.01                |
| intrinsic component of peroxisomal membrane  | GO:0031231    | 6                    | 1                               | 0.05                |
| intrinsic component of external side of plasma membrane | GO:0031233 | 6 | 1 | 0.05 |
| anchored component of external side of plasma membrane | GO:0031362 | 5 | 1 | 0.04 |
| Node name                                                          | Node ID      | No. of genes in node | No. of significant genes in node | Uncorrected p-value |
|-------------------------------------------------------------------|--------------|----------------------|----------------------------------|---------------------|
| Dbf4-dependent protein kinase complex                             | GO:0031431  | 1                    | 1                                | 0.01                |
| microbody membrane                                               | GO:0031903  | 22                   | 2                                | 0.01                |
| proton-transporting V-type ATPase complex                         | GO:0033176  | 6                    | 1                                | 0.05                |
| nuclear periphery                                                | GO:0034399  | 33                   | 2                                | 0.03                |
| centriolar satellite                                              | GO:0034451  | 33                   | 2                                | 0.03                |
| GID complex                                                       | GO:0034657  | 2                    | 1                                | 0.02                |
| endoplasmic reticulum chaperone complex                          | GO:0034663  | 4                    | 1                                | 0.03                |
| extrinsic component of endoplasmic reticulum membrane            | GO:0042406  | 4                    | 1                                | 0.03                |
| lamellar body                                                     | GO:0042599  | 4                    | 1                                | 0.03                |
| receptor complex                                                  | GO:0043235  | 108                  | 4                                | 0.01                |
| extracellular region part                                         | GO:0044421  | 916                  | 13                               | 0.03                |
| cytoskeletal part                                                 | GO:0044430  | 438                  | 8                                | 0.03                |
| microbody part                                                    | GO:0044438  | 40                   | 2                                | 0.04                |
| peroxisomal part                                                  | GO:0044439  | 40                   | 2                                | 0.04                |
| plasma membrane part                                              | GO:0044459  | 686                  | 11                               | 0.02                |
| contractile ring                                                  | GO:0070938  | 2                    | 1                                | 0.02                |
| cell periphery                                                    | GO:0071944  | 1303                 | 17                               | 0.03                |
| cation-transporting ATPase complex                                | GO:0090533  | 5                    | 2                                | 0                   |
| ATPase dependent transmembrane transport complex                  | GO:0098533  | 6                    | 2                                | 0                   |
| side of membrane                                                  | GO:0098552  | 138                  | 4                                | 0.03                |
| ATPase complex                                                    | GO:1904949  | 6                    | 2                                | 0                   |
| cellular protein localization                                     | GO:0034613  | 553                  | 1                                | 0.04                |
| cellular macromolecule localization                              | GO:0070727  | 557                  | 1                                | 0.04                |

**Genes involved in cold adaptation**

Next, we explored whether genes previously described to evolve differently in other cold adapted species are evolving faster or slower along the lineage to Steller’s sea cows. We used the list of genes collected by Yudin and colleagues that comprises of 4,380 genes selected in at least one of the following species: arctic fox, Yakutian horse, woolly mammoth, polar bear, minke whale, and human populations adapted to frigid environments (73). The curated list comprises genes under faster evolution in the cold-adapted species, 416 of which were present in at least two species. We compared the dataset to the 197 genes that we identified as evolving faster in the Steller’s sea cow lineage. We found that 51 genes with faster evolution rate in the Steller’s sea cow are selected in other cold-adapted species as well (hypergeometric test, p-value < 0.0001) (Supplementary Table S11).

Intriguingly, we identified four genes involved in cold adaptation evolving under natural selection in Steller’s sea cow, mammoth (74), and polar bear (75): RUNDC1, MRTO4, MKKS, and PLEKHG6. To our knowledge, the functions of RUNDC1 and MRTO4 have not yet been determined and appeared in the list of genes associated with cold adaptation only because of species-overlapping signatures of selection (73). Conversely, mutations in MKKS, the McKusick-
Kaufman Syndrome gene, are known to cause two distinct phenotypes in humans: MKKS and Bardet-Biedl Syndrome 6 (BBS6). MKKS is characterized by genitourinary malformations and polydactyly, while BBS6 includes polydactyly, kidney defects, but also obesity and delayed development among its symptoms. Because mice lacking *Mkks* are obese in addition to other phenotypic features typical of BBS, it has been hypothesized that complete loss of *Mkks* leads to BBS6 including obesity, while some mutations may cause MKKS (76). The functional advantage of *MKKS* selection in Steller’s sea cow is not directly evident, although it is tempting to speculate that this gene may be relevant to the enhanced adipose accumulation in cold environments. The function of *PLEKHG6*, or pleckstrin homology domain-containing family G member 6, is not fully understood, but its expression is positively correlated with lipid droplet size and is up-regulated in adipocytes specialized for lipogenesis (77). *PLEKHG6* is up-regulated in brown adipose tissue lacking the transcription factor *IRF4* (78), which has been shown to display reduced adaptive thermogenesis compared to the wild type (79). Although further analyses will be necessary to test this hypothesis, the upregulation of *PLEKHG6* may be a compensatory mechanism that allows better thermogenesis. This could be even more relevant, since *UCP1*, regarded as essential for NST, is absent in Steller’s sea cow and mammoth (25).

**Supplementary Table S11.**

Genes with signs of faster evolution in Steller’s sea cow and other arctic-adapted species. Genes under different evolution in at least two other species are marked in red.

| Gene ID | Species with faster evolution | Gene ID | Species with faster evolution |
|---------|-------------------------------|---------|-------------------------------|
| PLEKHG6 | Mammoth                       | SRP68   | Mammoth                       |
| RRP12   | Mammoth                       | PDILT   | Mammoth                       |
| MRT04   | Mammoth                       | DOLK    | Mammoth                       |
| OVGP1   | Mammoth                       | ACSF3   | Mammoth                       |
| GANAB   | Mammoth                       | ALS2CL  | Mammoth                       |
| RASD1   | Mammoth                       | TSH2    | Mammoth                       |
| VPS29   | Mammoth                       | C1orf116| Mammoth                       |
| PERP    | Mammoth                       | RFX6    | Mammoth                       |
| EHHADH  | Mammoth                       | SH2D5   | Mammoth                       |
| GJA3    | Mammoth                       | RAD54B  | Mammoth                       |
| RIOK1   | Mammoth                       | FAN1    | Mammoth                       |
| MKKS    | Mammoth                       | RUNDC1  | Mammoth                       |
| RSAD1   | Mammoth                       | PTX4    | Mammoth                       |
| APTX    | Mammoth                       | PLEKHG6 | Polar bear                    |
| TGS1    | Mammoth                       | EHD3    | Polar bear                    |
| DNA2    | Mammoth                       | MRT04   | Polar bear                    |
| CDH24   | Mammoth                       | KRT18   | Polar bear                    |
| GAS8    | Mammoth                       | MKKS    | Polar bear                    |
| SCYL1   | Mammoth                       | SERINC3 | Polar bear                    |
SM4: Demographic history reconstruction

Prehistoric population estimates

Prehistoric Steller’s sea cow population estimates for regions beyond Bering Island were calculated by extrapolating the number of sea cows per km² of habitat at Bering Island to the km² of habitat (x) in any region of interest.

Habitat data was obtained from GEBCO (general bathometric chart of the oceans; https://www.gebco.net/data_and_products/gridded_bathymetry_data/).

The number of Steller’s sea cows at Bering Island was around 1,000 animals (2). We calculated the area between shoreline and 20 m depth contour (approximate depth limit for kelp growth) as a proxy for sea cow habitat. The estimated area for Bering Island is 429 km².

From this we obtain the following estimates:

1. For all Alaska:
   - Area of habitat = 42,731 km²
   - Population estimate = (42,731 km² / 429 km²) * 1,000 = 99,600 sea cows

2. For all North America (Alaska to California):
   - Area of habitat = 57,383 km²
   - Population estimate = (57,383 km² / 429 km²) * 1,000 = 133,760 sea cows

3. For all North America and Russia (Kamchatka and Kurile Islands)
   - Area of habitat = 87,581 km²
   - Population estimate = (87,581 km² / 429 km²) * 1,000 = 204,150 sea cows

| Gene ID | Species with faster evolution | Gene ID | Species with faster evolution |
|---------|-------------------------------|---------|-------------------------------|
| HHIPL2  | Mammoth                       | ALOXE3  | Polar bear                    |
| TCTE1   | Mammoth                       | SLITRK6 | Polar bear                    |
| CNDPI   | Mammoth                       | SMYD4   | Polar bear                    |
| NMT2    | Mammoth                       | RUNDC1  | Polar bear                    |
| ME1OB   | Mammoth                       | OR8B3   | Polar bear                    |
| NIPAL1  | Mammoth                       | BVES    | Whale                         |
| COG1    | Mammoth                       |         |                               |
Inference of effective population size ($N_e$)

**Mutation rate**

To estimate the mutation rate in Steller’s sea cow, we used reads that were aligned to both dugong and manatee from the two ~15× individuals. Using the snpAD output, we filtered positions with coverage ≥ 5× and < 50×, GQ ≥ 20. To estimate the mutation rate, we counted only polymorphic sites that were fixed in both Steller’s sea cow individuals and different from manatee and dugong, respectively. The mutation rate per nucleotide per year was calculated as: $\mu = \frac{\text{no.ofderivedsites}}{\text{no.ofcoveredsites} \cdot 2t}$, where $t$ is the divergence time between the Steller’s sea cow and dugong (28.6 million years ago (7)), or manatee (41.6 million years ago (7)), respectively. Thus, we computed:

- for dugong: $\mu = \frac{30,577,847/2,309,546,751}{2 \cdot 28.6 \times 10^6} = 2.315 \times 10^{-10}$
- for manatee: $\mu = \frac{24,419,358/1,342,627,473}{2 \cdot 41.6 \times 10^6} = 2.186 \times 10^{-10}$

The mean estimated mutation rate for Steller’s sea cow is 0.225e-9, which is comparable to that of woolly mammoths (80).

**$N_e$ estimation based on pairwise sequentially Markovian coalescent (PSMC) method**

PSMC considers the density of heterozygous sites across the diploid genome from an individual to infer the distribution of the two alleles across all chromosomes as a function of time to the most recent common ancestor (49). This is then used to estimate effective population size ($N_e$) variations covering a time span of thousands of generations. Sirensians reach sexual maturity in 3–10 years (https://animaldiversity.org/accounts/Sirenia/); for comparison we considered a generation time of 3, 5, 6, and 10 years for Steller’s sea cow (Supplementary Fig. S6). PSMC relies on variant calling methods that are not based on population frequency and without assumption of Hardy–Weinberg equilibrium. We used samtools mpileup (44) to call variants for autosomal dugong scaffolds ≥ 100 kbp. The variants were inputted into PSMC with the (-p) parameter set to “4 + 25 × 2 + 4 + 6” and 100 bootstraps were performed to ensure more reliable accuracy of the demographic history trajectory. While the PSMC method has been shown to produce reliable estimates for simulated data in the distant past (> 10,000 years), its accuracy for time periods closer to present is not guaranteed (49). Based on the $N_e$ trajectory, the population decline of Steller’s sea cow began around 0.5 million years ago, at a time when the North Pacific area could not have been inhabited by humans. Generation time does not influence the overall trajectory or timeline, but shows an effect on the initial estimate for the effective population size (Supplementary Fig. S6).
Supplementary Fig. S6.

PSMC plots of the three Steller’s sea cow individuals considering different generation times (sirenians reach sexual maturity in 3–10 years [https://animaldiversity.org/accounts/Sirenia/]). The generation time shows a negative correlation with the estimated effective population size. Thus, the highest estimated effective population size corresponds to a generation time of 3 years. The trajectory of population size and the time scale are not affected by generation time.

Sex determination of the ~15×-coverage individuals

To determine the sex of the two ~15×-covered individuals, we calculated the ratio of the average coverage of the X chromosome to the average coverage of the autosomes. To this end, we initially determined which scaffolds from the dugong assembly correspond to the X chromosome based on a blast alignment of genes residing on the human chromosome X (GRCh38.p13). This resulted in 153 scaffolds with a total length of 201.3 million bp (Supplementary Table S12). The relative length of the X chromosome in the Indian elephant compared to the length of the entire genome is approximately 5.633 % (81); this suggests that for dugong the X-chromosome length would be approximately 181.4 million bp. The sum of the considered scaffolds is slightly larger, but because this represents about 10 % of the actual X-chromosome sequence, the ratio between X-to-autosomes will not be heavily influenced. This ratio is expected to be 1 in a female and 0.5
in a male. We obtained ratios of 0.93 and 1.23 (14.68$\times$ and 19.17$\times$ compared to the average 15.86$\times$ and 15.63$\times$), which suggests that both individuals are females and heterozygosity estimates can be calculated across all scaffolds. To confirm this result, we also checked whether any reads mapped to the SRY or ZFY dugong genes located on the Y chromosome, and, as expected, we did not identify Steller’s sea cow reads corresponding to those regions.

Supplementary Table S12.

Scaffolds from the de novo dugong assembly assigned to chromosome X.

| Dugong scaffold ID | Scaffold length (bp) | Mean coverage Ind01 | Mean coverage Ind02 |
|--------------------|----------------------|----------------------|----------------------|
| scaffold7          | 15,224,240           | 16.11                | 20.28                |
| scaffold18         | 17,251,047           | 14.65                | 17.50                |
| scaffold37         | 14,370,710           | 16.40                | 18.14                |
| scaffold79         | 5,595,303            | 16.84                | 18.56                |
| scaffold88         | 8,460,835            | 17.58                | 19.08                |
| scaffold91         | 6,694,011            | 17.32                | 20.56                |
| scaffold108        | 4,050,669            | 16.27                | 18.37                |
| scaffold141        | 3,630,362            | 15.57                | 18.38                |
| scaffold156        | 8,432,348            | 16.15                | 21.90                |
| scaffold198        | 3,087,677            | 15.33                | 19.69                |
| scaffold201        | 4,768,493            | 14.90                | 19.27                |
| scaffold239        | 3,594,916            | 16.23                | 21.99                |
| scaffold298        | 3,001,275            | 16.55                | 21.79                |
| scaffold347        | 9,823,323            | 15.73                | 18.25                |
| scaffold364        | 4,621,530            | 16.39                | 18.92                |
| scaffold376        | 1,882,354            | 15.47                | 18.62                |
| scaffold385        | 1,847,155            | 13.03                | 16.76                |
| scaffold398        | 1,811,345            | 15.02                | 18.66                |
| scaffold416        | 1,742,563            | 16.84                | 20.26                |
| scaffold422        | 2,074,037            | 14.52                | 17.07                |
| scaffold426        | 1,726,953            | 12.26                | 16.77                |
| scaffold478        | 1,925,199            | 14.09                | 18.32                |
| scaffold479        | 1,575,312            | 0.07                 | 0.06                 |
| scaffold500        | 1,522,886            | 16.52                | 21.68                |
| scaffold537        | 1,473,645            | 15.31                | 19.33                |
| scaffold546        | 1,410,123            | 16.34                | 21.47                |
| scaffold562        | 1,638,642            | 17.08                | 20.92                |
| scaffold584        | 1,306,290            | 12.11                | 16.93                |
| Dugong scaffold ID | Scaffold length (bp) | Mean coverage Ind01 | Mean coverage Ind02 |
|-------------------|---------------------|---------------------|---------------------|
| scaffold586      | 1,471,532           | 14.93               | 20.57               |
| scaffold614      | 1,242,202           | 14.76               | 18.34               |
| scaffold619      | 1,228,465           | 13.38               | 17.10               |
| scaffold623      | 1,217,361           | 15.02               | 19.56               |
| scaffold630      | 1,196,204           | 14.03               | 18.40               |
| scaffold631      | 1,195,602           | 12.51               | 15.42               |
| scaffold637      | 1,189,222           | 18.02               | 20.49               |
| scaffold647      | 1,157,931           | 18.13               | 21.19               |
| scaffold658      | 1,148,743           | 13.37               | 18.30               |
| scaffold662      | 2,383,847           | 11.99               | 16.00               |
| scaffold675      | 1,115,131           | 12.01               | 16.01               |
| scaffold747      | 978,951             | 12.36               | 17.04               |
| scaffold758      | 965,813             | 15.81               | 19.13               |
| scaffold759      | 1,178,196           | 15.64               | 19.20               |
| scaffold769      | 949,168             | 12.31               | 14.83               |
| scaffold779      | 935,908             | 14.25               | 19.23               |
| scaffold783      | 931,651             | 16.44               | 21.70               |
| scaffold795      | 912,243             | 17.55               | 22.25               |
| scaffold800      | 906,131             | 14.01               | 21.05               |
| scaffold803      | 901,153             | 16.04               | 20.29               |
| scaffold811      | 923,461             | 0.05                | 0.04                |
| scaffold816      | 1,202,943           | 12.02               | 16.14               |
| scaffold825      | 876,245             | 16.81               | 16.94               |
| scaffold844      | 1,731,182           | 13.05               | 17.70               |
| scaffold862      | 960,403             | 0.06                | 0.05                |
| scaffold869      | 1,106,877           | 16.02               | 20.63               |
| scaffold883      | 1,286,841           | 15.45               | 20.16               |
| scaffold972      | 666,031             | 15.04               | 18.92               |
| scaffold980      | 654,432             | 11.17               | 15.38               |
| scaffold984      | 1,248,677           | 17.65               | 22.86               |
| scaffold992      | 942,668             | 17.26               | 21.18               |
| scaffold995      | 631,532             | 17.34               | 22.04               |
| scaffold1001     | 937,064             | 13.66               | 18.52               |
| scaffold1005     | 620,131             | 13.40               | 19.80               |
| scaffold1012     | 718,877             | 13.50               | 19.35               |
| scaffold1018     | 803,886             | 14.80               | 19.16               |
| scaffold1019     | 612,082             | 11.78               | 14.25               |
| Dugong scaffold ID | Scaffold length (bp) | Mean coverage Ind01 | Mean coverage Ind02 |
|--------------------|---------------------|---------------------|---------------------|
| scaffold1037       | 591,429             | 16.17               | 21.33               |
| scaffold1067       | 1,423,584           | 13.14               | 18.08               |
| scaffold1109       | 656,714             | 15.00               | 20.55               |
| scaffold1112       | 1,404,205           | 16.32               | 19.38               |
| scaffold1114       | 522,711             | 17.27               | 20.01               |
| scaffold1117       | 537,854             | 15.76               | 19.36               |
| scaffold1121       | 717,088             | 15.95               | 20.69               |
| scaffold1123       | 513,832             | 11.26               | 15.78               |
| scaffold1125       | 913,104             | 16.31               | 21.07               |
| scaffold1126       | 713,031             | 16.21               | 21.03               |
| scaffold1131       | 508,410             | 15.13               | 19.92               |
| scaffold1136       | 536,882             | 13.78               | 17.87               |
| scaffold1145       | 498,065             | 15.43               | 19.24               |
| scaffold1160       | 482,206             | 14.44               | 18.88               |
| scaffold1164       | 472,738             | 14.29               | 18.64               |
| scaffold1166       | 471,438             | 14.19               | 18.39               |
| scaffold1168       | 493,835             | 13.75               | 17.69               |
| scaffold1222       | 430,380             | 15.24               | 20.21               |
| scaffold1227       | 510,886             | 14.99               | 19.77               |
| scaffold1235       | 420,963             | 15.53               | 20.42               |
| scaffold1240       | 416,642             | 16.01               | 18.48               |
| scaffold1249       | 447,741             | 12.32               | 17.10               |
| scaffold1255       | 444,952             | 14.24               | 16.50               |
| scaffold1260       | 484,526             | 0.03                | 0.03                |
| scaffold1271       | 396,544             | 17.23               | 21.06               |
| scaffold1272       | 413,669             | 12.66               | 17.40               |
| scaffold1302       | 372,349             | 18.03               | 24.33               |
| scaffold1314       | 365,108             | 13.41               | 16.03               |
| scaffold1318       | 363,403             | 15.61               | 21.56               |
| scaffold1323       | 358,456             | 12.85               | 14.98               |
| scaffold1328       | 357,130             | 17.87               | 21.61               |
| scaffold1343       | 351,100             | 14.51               | 19.44               |
| scaffold1344       | 350,306             | 13.18               | 19.87               |
| scaffold1351       | 506,834             | 15.21               | 21.80               |
| scaffold1354       | 357,464             | 16.59               | 20.19               |
| scaffold1360       | 340,258             | 13.23               | 19.96               |
| scaffold1369       | 401,922             | 15.17               | 19.94               |
| Dugong scaffold ID | Scaffold length (bp) | Mean coverage Ind01 | Mean coverage Ind02 |
|-------------------|---------------------|---------------------|---------------------|
| scaffold1401      | 392,625             | 12.69               | 18.77               |
| scaffold1407      | 426,720             | 15.11               | 20.00               |
| scaffold1467      | 294,254             | 12.90               | 18.51               |
| scaffold1480      | 271,984             | 13.63               | 18.62               |
| scaffold1508      | 271,670             | 17.67               | 23.62               |
| scaffold1519      | 431,402             | 16.12               | 20.89               |
| scaffold1528      | 249,864             | 16.46               | 21.97               |
| scaffold1536      | 269,991             | 15.36               | 20.43               |
| scaffold1538      | 256,533             | 13.35               | 19.05               |
| scaffold1575      | 231,741             | 11.64               | 15.76               |
| scaffold1576      | 231,266             | 16.45               | 19.98               |
| scaffold1595      | 229,837             | 12.57               | 17.31               |
| scaffold1596      | 221,616             | 16.23               | 21.45               |
| scaffold1613      | 216,572             | 14.82               | 18.61               |
| scaffold1620      | 213,679             | 18.89               | 21.90               |
| scaffold1625      | 222,770             | 12.35               | 17.48               |
| scaffold1637      | 208,478             | 0.04                | 0.03                |
| scaffold1658      | 199,242             | 15.71               | 20.82               |
| scaffold1676      | 456,324             | 12.82               | 17.68               |
| scaffold1707      | 183,206             | 15.19               | 17.07               |
| scaffold1747      | 171,000             | 17.38               | 25.86               |
| scaffold1750      | 170,304             | 17.84               | 24.05               |
| scaffold1799      | 155,872             | 14.39               | 16.61               |
| scaffold1888      | 133,363             | 18.62               | 20.70               |
| scaffold1898      | 130,930             | 16.90               | 25.20               |
| scaffold1960      | 118,259             | 17.07               | 25.72               |
| scaffold1962      | 117,884             | 14.63               | 20.18               |
| scaffold1975      | 114,250             | 12.25               | 17.77               |
| scaffold2006      | 107,735             | 15.87               | 23.04               |
| scaffold2019      | 105,969             | 13.76               | 18.20               |
| scaffold2040      | 120,537             | 14.52               | 19.58               |
| scaffold2085      | 94,923              | 15.54               | 18.95               |
| scaffold2097      | 93,100              | 17.16               | 22.67               |
| scaffold2131      | 101,433             | 15.67               | 20.15               |
| scaffold2168      | 82,730              | 15.72               | 17.82               |
| scaffold2204      | 78,653              | 16.22               | 20.00               |
| scaffold2216      | 76,809              | 13.63               | 20.97               |
| Dugong scaffold ID | Scaffold length (bp) | Mean coverage Ind01 | Mean coverage Ind02 |
|-------------------|---------------------|---------------------|---------------------|
| scaffold2246      | 73,297              | 16.22               | 22.15               |
| scaffold2260      | 71,345              | 18.00               | 25.03               |
| scaffold2323      | 104,372             | 22.67               | 30.17               |
| scaffold2528      | 49,333              | 18.47               | 24.18               |
| scaffold2649      | 42,734              | 19.03               | 26.44               |
| scaffold2671      | 41,606              | 15.03               | 23.12               |
| scaffold2688      | 40,587              | 17.04               | 26.93               |
| scaffold2907      | 33,307              | 13.75               | 21.16               |
| scaffold3012      | 31,060              | 14.86               | 22.60               |
| scaffold3091      | 48,139              | 12.26               | 18.12               |
| scaffold3181      | 26,663              | 11.93               | 18.70               |
| scaffold3542      | 20,015              | 15.95               | 26.09               |
| scaffold3840      | 16,285              | 16.52               | 25.45               |
| scaffold4549      | 10,962              | 16.57               | 18.26               |
| Total length      | 201,283,018         |                     |                     |

Population diversity estimates

To determine the level of diversity over time in the sampled Steller’s sea cow populations, we performed pairwise comparisons of individuals based on their radiocarbon dating results (Supplementary Table S1). We used Consensify (51) to generate a consensus pseudohaploid genome sequence. The method controls for errors introduced by differential sequencing coverage. For each position of the dugong genome, three bases were selected from the Steller’s sea cow’s read stack. If ≥ 2 out of the selected three reads agree, the base is retained. If only two reads are present and in agreement, the base is also retained. If no two reads agree, an “N” is entered for that position. If coverage is < 2, or above twice the average coverage for the library (Supplementary Table S1), then an N is entered for that position. Thus, only libraries with an average coverage > 2 could be included in the pairwise comparisons. Pairwise comparisons were performed for individuals dated to the same time period. We excluded every position which contained an N in any of the two individuals in the pairwise comparison and counted the number of differences within non-overlapping blocks of 50 kbp. For the ~15× individuals we included all scaffolds > 50 kbp, since we could test that they were females. For the low-coverage individuals we included scaffolds > 50 kbp and excluded scaffolds assigned to sex chromosomes, since their sex could not be assigned. We included 10,000 randomly selected blocks of 50 kbp in the pairwise comparisons and used the R ggplot2 density function to plot the differences over these blocks (Fig. 3B) (82).
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