REPORT

OPEN ACCESS

www.the-innovation.org

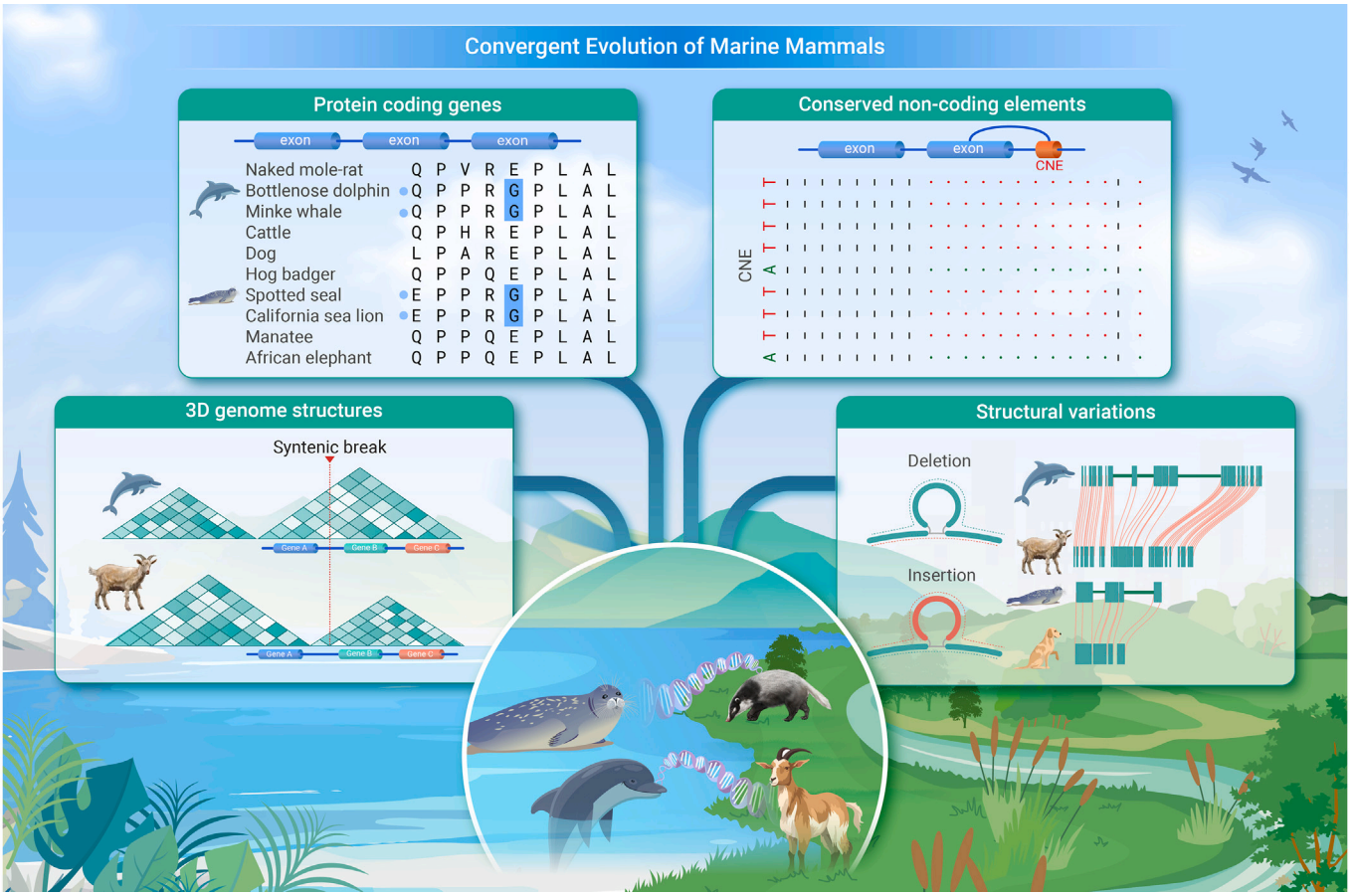
Multi-level genomic convergence of secondary aquatic adaptation in marine mammals

Shixia Xu,[1,5,*] Lei Shan,[1,5] Ran Tian,[1,5] Zhenpeng Yu,[1,5] Di Sun,[1] Zhenhua Zhang,[1] Inge Seim,[3,4] Ming Zhou,[1] Linxia Sun,[1] Na Liang,[1] Qian Zhang,[1] Simin Chai,[2] Daiqing Yin,[2] Luoying Deme,[1] Tianzhen Wu,[1] Yongjie Chen,[1] Zhikang Xu,[1] Yu Zheng,[1] Wenhua Ren,[1] and Guang Yang[1,2,*]

*Correspondence: xushixia@njnu.edu.cn (S.X.); gyang@njnu.edu.cn (G.Y.)

Received: June 18, 2024; Accepted: January 1, 2025; Published Online: March 3, 2025; https://doi.org/10.1016/j.xinn.2025.100798

GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- High-quality chromosome-level genome assemblies were produced for two marine mammals and one terrestrial relative.
- Convergent evolution of secondary aquatic adaptation is evident in marine mammals.
- Convergent amino acid changes in P378L (*APPL1*) and E71G (*NEIL1*) enhance lipid accumulation and reduce cervical cancer cell proliferation.
- Genetic variations in non-coding elements contribute to cancer suppression, blubber thickening, and neuronal traits.

OPEN ACCESS

REPORT

The Innovation

Multi-level genomic convergence of secondary aquatic adaptation in marine mammals

Shixia Xu,[1,5,*] Lei Shan,[1,5] Ran Tian,[1,5] Zhenpeng Yu,[1,5] Di Sun,[1] Zhenhua Zhang,[1] Inge Seim,[3,4] Ming Zhou,[1] Linxia Sun,[1] Na Liang,[1] Qian Zhang,[1] Simin Chai,[2] Daiqing Yin,[2] Luoying Deme,[1] Tianzhen Wu,[1] Yongjie Chen,[1] Zhikang Xu,[1] Yu Zheng,[1] Wenhua Ren,[1] and Guang Yang[1,2,*]

[1]Jiangsu Key Laboratory for the Biodiversity Conservation and Sustainable Utilization in the Middle and Lower Reaches of Yangtze River Basin, College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

[2]Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 511458, China

[3]Integrative Biology Laboratory, College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

[4]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, QLD 4002, Australia

[5]These authors contributed equally

*Correspondence: xushixia@njnu.edu.cn (S.X.); gyang@njnu.edu.cn (G.Y.)

Received: June 18, 2024; Accepted: January 1, 2025; Published Online: March 3, 2025; https://doi.org/10.1016/j.xinn.2025.100798

Citation: Xu S., Shan L., Tian R., et al., (2025). Multi-level genomic convergence of secondary aquatic adaptation in marine mammals. The Innovation **6(3)**, 100798.

Marine mammals provide a valuable model for studying the molecular basis of convergent evolution during secondary aquatic adaptation. Using multi-omics data and functional experiments, including CRISPR-Cas9 mouse models and luciferase reporter assays, this study explored the molecular mechanisms driving this transition across coding regions, regulatory elements, and genomic architecture. Convergent amino acid substitutions in *APPL1*^{P378L} and *NEIL1*^{E71G} were found to promote lipid accumulation and suppress cancer cell proliferation, likely contributing to the evolution of extensive blubber layers and cancer resistance. Convergently evolved conserved non-exonic elements (CNEs) and lineage-specific regulatory variations were shown to influence the activity of nearby genes (e.g., *NKX3-2*, *SOX9*, *HAND2*), shaping cetacean limb phenotypes. Additionally, convergent shifts in topologically associating domains (TADs) across cetaceans and pinnipeds were implicated in the regulation of *ASXL3* and *FAM43B* expression, playing a role in the formation of thickened blubber layers and mitigating cancer susceptibility. Structural variations within conserved TADs were associated with the expression of neuronal genes, including *NUP153* and *ID4*, potentially driving cognitive and social adaptations. These findings provide novel insights into the molecular foundations of the convergent evolution of secondary aquatic adaptations in mammals.

INTRODUCTION

The independent evolutionary transition from land to water by the ancestors of cetaceans (whales, dolphins, and porpoises), pinnipeds (seals, sea lions, and walruses), and sirenians (manatees and dugongs) represents one of the most profound shifts in vertebrate history.[1] Despite their distinct evolutionary trajectories, these marine mammals exhibit strikingly similar morphological and physiological adaptations. These include paddle-like limbs, thick insulating blubber, hydrodynamic body shapes, and advanced cognitive abilities associated with complex social structures and enhanced brain development, particularly neocortical organization. Such traits exemplify phenotypic convergence, an adaptive response to the demands of aquatic life. A central question in marine mammal evolution is the extent to which these convergent phenotypes are underpinned by molecular convergence. Foote et al. utilized genome-wide analyses to investigate the genetic signatures of molecular convergence in marine mammals.[2] Their findings, supported by subsequent studies,[3,4] revealed that adaptive convergence at the amino acid level is relatively infrequent. Furthermore, these amino acid changes do not necessarily align with phenotypic convergence, suggesting that molecular convergence may arise in different genomic regions or across distinct levels of genomic organization, highlighting the complexity of these evolutionary processes.

Accumulating evidence has indicated that variations in conserved non-exonic elements (CNEs) are significant contributors to morphological convergence. Such variations have been implicated in key evolutionary changes, including the loss of flight in paleognathous birds,[5] the loss of limbs in squamates,[6] and the evolution of mammalian vision.[7,8] Proximal to the developmental or differentiation-associated genes, CNEs frequently act as enhancers, serving as *cis*-regulatory modules orchestrating the precise spatiotemporal regulation of gene expression.[9] Alterations in rapidly evolving CNEs can modify their regulatory functions, potentially driving the emergence of convergent phenotypes. However, the role of CNE evolution in shaping convergent phenotypes in marine mammals remains poorly understood.[10]

Beyond sequence-level changes, chromosomal structural variations (SVs), such as syntenic breaks, may also contribute to a range of evolutionary phenomena.[11] Genomic rearrangements within the 3p21.31 tumor suppressor gene cluster, observed in distantly related rodent species from the suborders Hystricomorpha (e.g., naked mole rats, African crested porcupines) and Sciuromorpha (e.g., beavers, squirrels), have been associated with cancer resistance,[12] suggesting a potential convergent mechanism involving alterations in genomic architecture. These findings underscore the importance of investigating correlations between syntenic breaks, three-dimensional (3D) chromatin remodeling, and their potential roles in phenotypic convergence.[13] Currently, however, our understanding of such convergent events in the genome architecture of marine mammals remains limited.

To address this gap, our study assembled high-quality chromosomal genomes for two marine lineages, cetacean bottlenose dolphin (*Tursiops truncatus*) and pinniped spotted seal (*Phoca largha*), and one terrestrial relative, hog badger (*Arctonyx collaris*). High-resolution genome-wide chromatin interaction maps were reconstructed for the bottlenose dolphin, spotted seal, and two terrestrial relatives (hog badger and goat, *Capra hircus*), incorporating previously published goat genomic data. A comprehensive analysis of shared amino acid substitutions, candidate regulatory elements, and genome architecture was performed using multi-omics data, identifying a multi-level genomic convergence landscape for marine mammals undergoing secondary adaptation to aquatic life. Genetic changes were further linked to molecular functions and phenotypic traits, including the development of insulating blubber, aquatic locomotion, neuronal complexity, and cancer resistance. Collectively, these findings provide critical insights into the molecular and genomic mechanisms underpinning the secondary adaptation of marine mammals to life in water.

MATERIALS AND METHODS

Experimental model and subject details

All animal work was reviewed and approved by the Institute for Animal Use and Ethical Committees of Nanjing Normal University (approval no.: IACUC-20200501).

More detailed materials and methods related to this study are available in the supplemental information.

RESULTS

Genome assembly and divergence time estimation

Chromosome-level genome assemblies were generated for the bottlenose dolphin (*T. truncatus*), spotted seal (*P. largha*), and hog badger (*A. collaris*) using PacBio SMRT long-read sequencing, short-read sequencing, BioNano optical mapping, and Hi-C technology (Figure 1A; Table S1). The final assemblies were approximately 2.36, 2.41, and 2.61 Gb in size, respectively, with scaffold N50 lengths ranging from 115.7 Mb (bottlenose dolphin) to 179.7 Mb (spotted seal). Quality assessments showed that over 97.8% of short reads mapped to their respective genomes, achieving consensus quality scores exceeding 26.3 (Table S2). The genome assemblies showed higher contiguity and accuracy than previously reported assemblies (bottlenose dolphin: GCA_011762595.1, Hawaiian monk seal: GCA_002201575.2) (Table S3).[14] The assemblies were anchored to pseudochromosomes, consistent with the expected karyotypes of

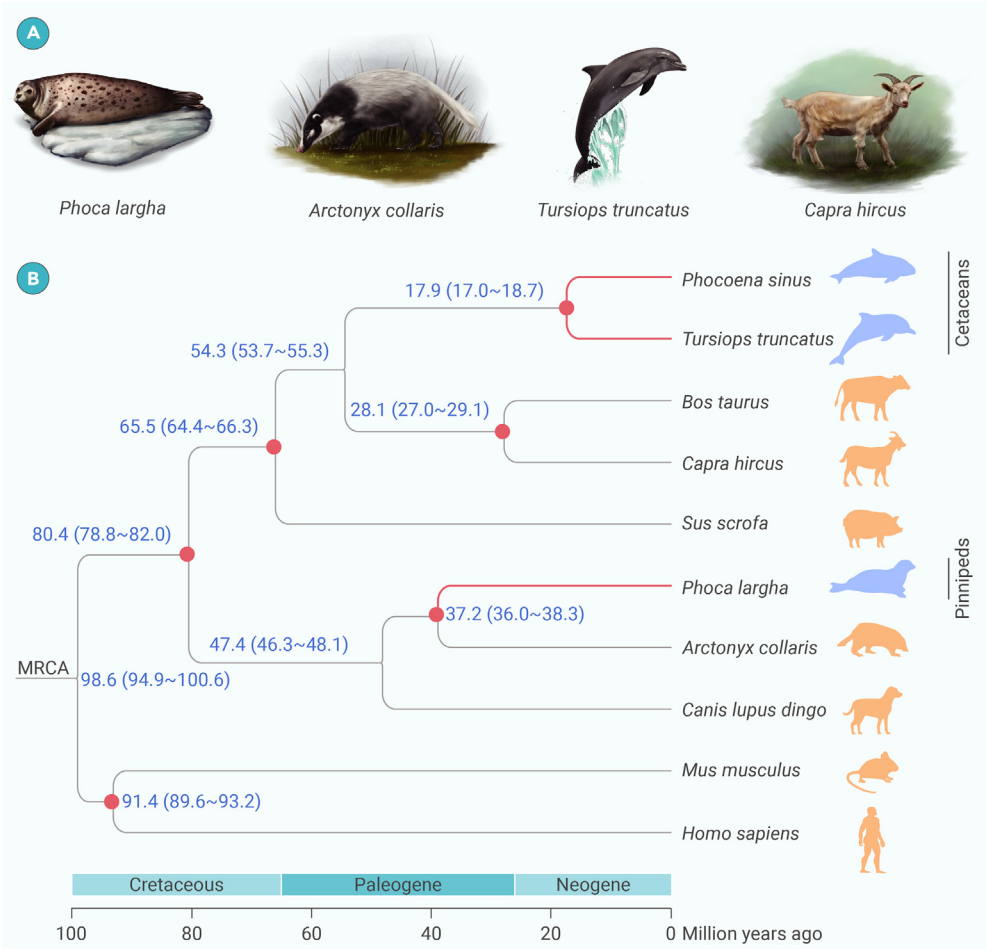


Figure 1. Genome assembly and phylogenetic relationships (A) Diagrams of four mammals newly sequenced in this study. The first three (from left to right) were *de novo* assembled, with Hi-C data generated only for the goat. (B) Phylogenetic relationships and divergence times of *Tursiops truncatus*, *Capra hircus*, and eight other mammalian genomes. Phylogenetic tree was reconstructed using RAxML, with 8425 shared one-to-one orthologous genes. Blue numbers correspond to divergence times between species, with confidence ranges shown in brackets. Red clades represent cetacean and pinniped lineages. Red circles represent fossil calibrations.

each species (bottlenose dolphin 2*n* = 44, spotted seal 2*n* = 32, and hog badger 2*n* = 44) (Figure S1). Additionally, Hi-C data for the goat (*C. hircus*) were generated to facilitate subsequent analyses of genomic architecture (Table S1).

Based on homology, *ab initio*, and transcriptome-based prediction models, 24,118, 21,069, and 21,567 protein-coding genes were identified in the bottlenose dolphin, spotted seal, and hog badger, respectively (Table S2). Gene assembly completeness was assessed using Benchmark Universal Single-Copy Orthologs analysis,[15] with 93.3%–95.7% of the 4104 highly conserved mammalian core proteins present (Table S4). Transposable elements accounted for 44.08%–49.10% of the genome (Table S5), comparable to that reported for other mammalian species (Figure S2).

In total, 8425 one-to-one orthologous genes were identified among the three sequenced mammals and seven other species with high-quality genomes. These genes were employed to construct a phylogenetic tree (Figure 1B), which showed strong congruence with previously constructed mammalian species trees.[16] Divergence time estimates placed the split between cetaceans (bottlenose dolphins and vaquitas) and even-toed ungulates (cattle and goats) at approximately 54.3 mya, while the divergence between spotted seals and hog badgers occurred around 37.2 mya. Both events were dated to the Paleogene period, consistent with previous studies.[16]

Convergent amino acid replacements in marine mammals

To identify candidate convergent amino acid substitutions in marine mammals, 4692 orthologous genes were analyzed using synteny-based alignments across 50 mammals, including 26 marine species (16 cetaceans, 9 pinnipeds, and 1 sirenian) and 24 terrestrial species (Table S6). Additional filtering against 106 terrestrial species from the OrthoMaM database[17] identified 82 genes with convergent amino acid substitutions specific to marine mammals, either among the three marine orders or between cetacean and pinniped lineages. These genes were enriched in Gene Ontology (GO) terms associated with cancer suppression, lipid metabolism, inflammatory response, and cell-cycle regulation. Among these genes, six (*ABCC10*, *ANKRD1*, *APPL1*, *ITGA8*, *NODAL*, and *NFIA*) exhibited convergent substitutions across all three marine mammal orders (Table S7), while eight lipid metabolism-related genes (*AASDH*, *ABCA12*, *ADCY3*, *APPL1*, *CPT2*, *GPAM*, *NFIA*, and *PRKAG3*) displayed substitutions in at least two marine mammal lineages (Table S7). Polymorphisms in *APPL1* and *ADCY3* have been associated with body mass index and fat distribution in individuals with type 2 diabetes,[18,19] with *APPL1* playing a critical role in adipocyte dynamics and homeostasis in mice.[20] Notably, a convergent amino acid substitution (P378L) near the pleckstrin homology domain of APPL1 was identified in cetaceans, manatees, and four pinnipeds residing in colder environments (Figure 2A). Functional analyses revealed that overexpression of the APPL1^{P378L} variant in the 3T3-L1 murine preadipocyte cell line markedly elevated lipid accumulation, as visualized by oil red O staining (Figures 2A and 2B). Furthermore, the expression of key adipogenesis markers, including peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein-α (CEBPα), and fatty acid binding protein 4 (FABP4), was also elevated in cells overexpressing *APPL1*^{P378L} compared to wild-type (WT) murine cells (Figure 2C). These findings suggest that the P378L substitution in *APPL1* promotes lipid accumulation and adipogenesis, facilitating the development of thickened blubber in marine mammals.

Convergent evolution was also observed in 27 cancer-associated genes, with six (*NEIL1*, *EME1*, *FAAP100*, *REV3L*, *SDE2*, and *WDR18*) shared between cetaceans and pinnipeds. These genes play pivotal roles in DNA repair processes, including excision of damaged DNA, maintenance of genomic stability, and tumor suppression (Figure 2E; Table S7). *NEIL1* is essential for DNA base excision repair, with previous research showing elevated levels of DNA damage in *Neil1* knockout mice.[21] In this study, a substantial convergent radical amino acid alteration (E71G) within the DNA glycosylase N-terminal domain of NEIL1 was identified in cetaceans and pinnipeds (Figure 2F). Functional assays demonstrated that *Neil1*^{E71G} mutant murine cells exhibited significantly reduced proliferation rates compared to WT cells, as assessed by 5-ethynyl-2′-deoxyuridine (EdU) assays (Figures 2G and 2H). These findings suggest that the *NEIL1*^{E71G} substitution may enhance DNA repair efficiency, contributing to the remarkable cancer resistance observed in marine mammals.

Convergent and lineage-specific variations in non-coding regulatory elements

Regulatory domains are posited to experience fewer pleiotropic constraints compared to protein-coding sequences,[22,23] making them more plausible drivers of convergent traits.[5] To identify convergent changes in non-coding elements among marine mammals, 16 cetaceans, 9 pinnipeds, and 1 sirenian were compared with 12 terrestrial mammals (Table S8). Using 38-way whole-genome alignments (WGAs), 333,341 mammalian CNEs were first identified, 1280 of which showed evidence of substitution rate shifts specific to marine mammals.

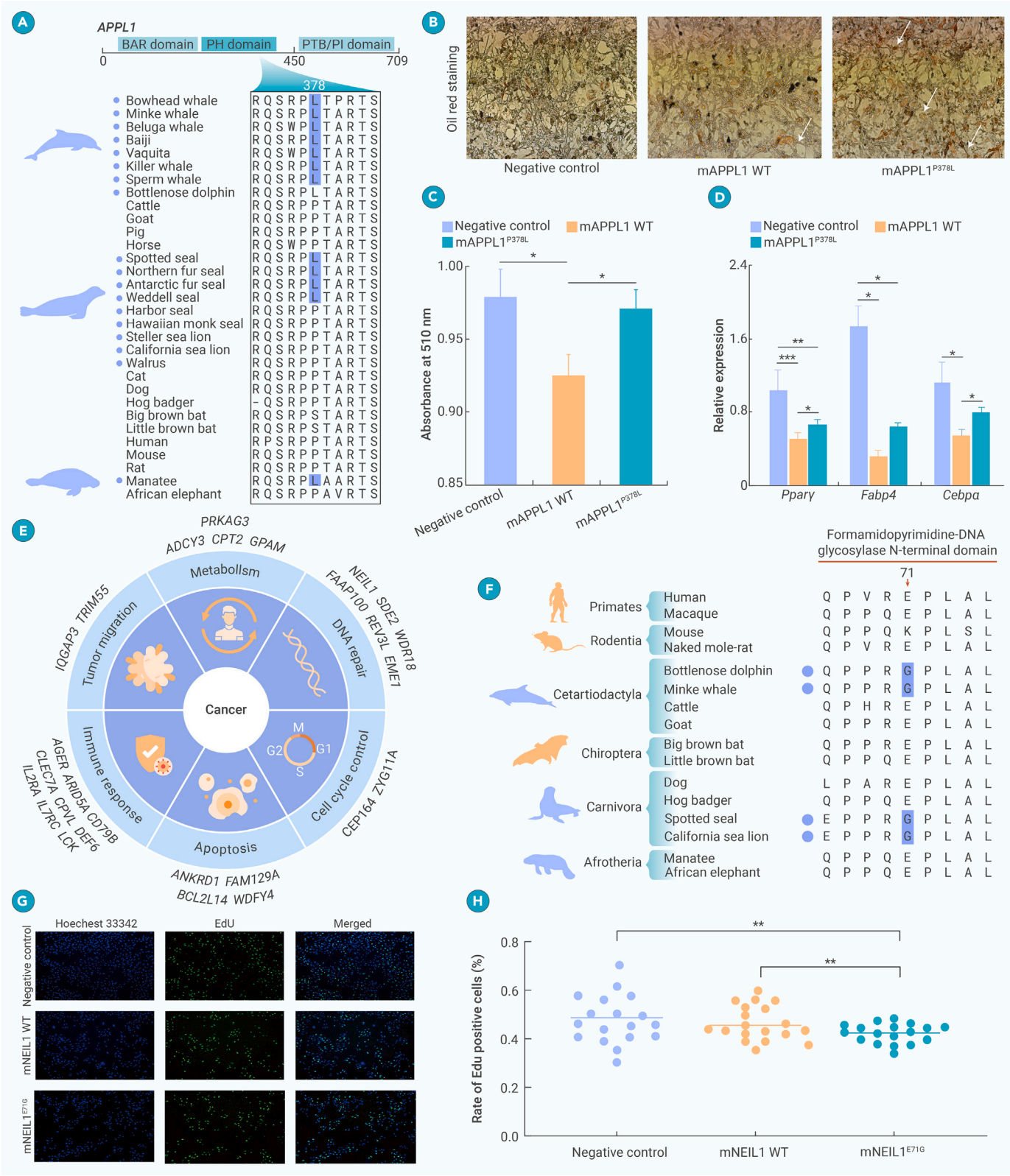


Figure 2. Convergent evolution of coding genes (A) Convergent amino acid changes in *APPL1* gene (blue) observed in cetaceans, manatees, and several pinnipeds. (B) Oil red O staining of 3T3-L1 adipocytes on day 8, with black arrows indicating lipid droplets. (C) Histogram showing lipid content extracted from oil red O-stained 3T3-L1 adipocytes using 100% isopropanol. (D) Expression of adipogenesis markers *PPARγ*, *FABP4*, and *CEBPα* in 3T3-L1 cells incubated with adipogenic cocktail for 8 days. Negative control, *mAPPL1* WT, and *mAPPL1*^{P378L} are in orange, blue, and green, respectively. (E) Cancer resistance-related genes in marine mammals, classified according to functional roles. (F) Sequence alignments showing convergent amino acid changes (blue) identified in *NEIL1* for cetaceans and pinnipeds. (G and H) EdU assays assessing proliferation of human HeLa cells transfected with wild-type mouse (*mNEIL1WT*) and mouse *NEIL1* containing a cetacean + pinniped convergent amino acid substitution (*mNEIL1*^{E71G}). Data are means ± standard errors of the mean (SEMs) of three independent experiments. $^{**}p < 0.01$ by t test.

These marine mammal-accelerated CNEs included 369 shared across all 3 marine mammalian orders, 835 shared between cetaceans and pinnipeds, 66 shared between cetaceans and sirenians, and 10 shared between pinnipeds and sirenians (Table S9). Functional enrichment analysis revealed that the 369 CNEs shared across marine mammal orders were significantly enriched in terms related to organ development, including skeletal system development (GO: 0001501), anatomical structure development (GO: 0048856), nervous system development (GO: 0007399), and animal organ development (GO: 0048513) (Table S10).

A total of 48 CNEs exhibiting marine mammal-specific convergent acceleration were identified proximal to genes associated with limb development, as determined using chromatin state datasets derived from mice (Figure 3A; Table S9). Key limb development genes proximal to these CNEs included *NKX3-2* (CNE300), *SOX9* (CNE1402), and *HAND2* (CNE457 and CNE458) (Figure 3A; Table S9), highlighting their potential regulatory significance. To investigate their functional roles, a subset of 10 limb development-related CNEs were randomly selected for *in vitro* dual luciferase reporter gene experiments to assess potential regulatory activity (Figure 3B). Among these, 8 of the 10 CNEs exhibited differential reporter activity compared to the luciferase controls, indicating modified regulatory functions, while 2 CNEs (CNE1505 and 634) showed no significant changes (Figure 3B). Notably, comparisons with mouse sequences revealed variable regulatory activities among marine mammal CNEs, demonstrating either increased (eg, CNE1402) or reduced (eg, CNE1536 in both dolphins and spotted seals, CNE300 in dolphins) functional activities. These functional variations in regulatory activity suggest potential alterations in the expression of target genes, such as *NKX3-2*, *SOX9*, and *HAND2*, which may influence limb developmental processes.

Analysis of 38-way WGAs revealed CNEs with convergent deletions exceeding 5 bp exclusively in marine mammal lineages, including cetaceans, pinnipeds, and sirenians. Extensive alignment of 120 mammalian genomes confirmed that these deletions were not attributable to sequencing gaps or assembly errors (https://bds.mpi-cbg.de/hillerlab/120MammalAlignment).[24] Interestingly, the deletions were not uniformly distributed across all marine mammal groups. A total of 271 CNEs contained short deletions shared by only 2 marine orders, including 64 between Cetacea and Pinnipedia, 151 between Cetacea and Sirenia, and 56 between Pinnipedia and Sirenia (Table S9). Subsequent functional enrichment analysis revealed significant enrichment in terms related to tissue development (GO: 0009888), system development (GO: 0048731), nervous system development (GO: 0007399), and tissue morphogenesis (GO: 0048729) (Table S11). Among these, a 9-bp deletion within an accelerated CNE (CNE1402), located 230 kb upstream of the *SOX9* gene, was shared by cetaceans and manatees (Figure S3A). As a key regulator of bone differentiation in limb mesenchymal cells,[25] *SOX9* has been implicated in limb malformations through changes in its upstream *cis*-regulatory elements.[26]

To explore the functional implications of this shared deletion, plasmids containing the bottlenose dolphin (Ttru_CNE1402) and spotted seal (Plar_CNE1402) sequences were constructed as representatives of cetacean and pinniped CNE1402 variants, respectively (Figure S3B). Luciferase assays revealed that these marine-specific CNEs exhibited higher regulatory activity compared to their mouse counterparts, suggesting that the 9-bp deletion may enhance *cis*-regulatory function (Figure S3B). These findings indicate that CNE1402 likely contributes to shaping limb phenotypes in marine mammals, with the shared 9-bp deletion potentially driving phenotypic convergence between fully aquatic cetaceans and sirenians.

Lineage-specific genomic variations in regulatory elements associated with limb development were identified, including a cetacean-specific 12-bp deletion within the accelerated CNE300 present in cetaceans, pinnipeds, and manatees (Figure 3C). CNE300 overlaps with a known human limb enhancer (hs259) and aligns with DNase sequencing (DNase-seq) and assay for transposase accessible chromatin-seq peak regions derived from mouse embryonic limb profiles (Figures 3C, 3D, and S4; Table S9). Luciferase assays revealed reduced enhancer activity for the cetacean CNE300 compared to equivalent mouse and spotted seal sequences (Figure 3B). Furthermore, analysis showed that CNE300 and its proximal gene *NKX3-2* were situated within the same topologically associating domain (TAD) across multiple species, including mice, bottlenose dolphins, and spotted seals. This spatial genomic organization suggests potential regulatory interactions between CNE300 and *NKX3-2*. The *NKX3-2* gene is thought to play a key role in chondrogenesis and functions downstream of the sonic hedgehog (SHH) signaling pathway. Suppression of SHH expression has been documented in the hindlimb bud of the pantropical spotted dolphin (*Stenella attenuata*).[27] We hypothesize that the deletion in cetacean CNE300 may play a role in hindlimb regression, acting in synergy with reduced SHH expression in late-stage embryonic development. However, further experimental studies are needed to ascertain the impact of these genetic variations on regulatory activities.

Four cetacean-specific deletions were identified in hindlimb enhancer A (HLEA), a regulatory element essential for TBX4 expression during hindlimb bud development (Figure S5).[28] These deletions, hallmarks of lineage-specific genomic variation, have been shown to reduce enhancer activity *in vitro*, as demonstrated by luciferase reporter assays.[29] To assess the functional implications of these mutations *in vivo*, a CRISPR-Cas9-mediated knockin (KI) mouse model was generated, incorporating the bottlenose dolphin HLEA sequence. Heterozygous KI/WT mice displayed distinct alterations in hindlimb morphology compared to WT mice, including broader tibiae and shorter patellae (Figures 3E and 3F). These findings suggest that cetacean-specific HLEA mutations affect distal bone development, consistent with patterns observed in previous research.[29] RNA-seq of hindlimb buds at embryonic stages E10.5, E11.5, and E12.5 revealed widespread transcriptional changes, with 979, 371, and 430 differentially expressed genes (DEGs) identified at each stage, respectively (Figure S6; Tables S12–S14), suggesting a substantial influence of the HLEA region on gene expression during early developmental phases. Subsequent functional enrichment analysis of these DEGs highlighted significant associations with limb development-related terms, including axonogenesis, muscle tissue development, and skeletal morphogenesis (Figure S7). Notably, several DEGs identified in this study are critical for limb formation, including *NKX3-1/3-2*, *BMP2*, *FGF3/6/7*, *HAND1/2*, and *PITX1* (Figures 3G and S8). Dysregulation or loss of these genes has been associated with severe limb truncations and malformations, highlighting their essential roles in limb development.[30] These results suggest that specific deletions in cetacean HLEA may have contributed to the observed loss of hindlimbs during secondary aquatic adaptation in marine mammals.

Chromosomal syntenic breaks and 3D genomic conformation

To investigate potential convergent chromosomal alterations and 3D genomic architecture, high-resolution genome-wide chromatin interaction maps were reconstructed for the bottlenose dolphin, spotted seal, hog badger, and goat. The initial contig-level assembly of the goat was sourced from a previous study.[31] Using Hi-C technology, approximately 1 billion clean reads were generated for each species, with biological replicates showing strong correlations ($R \geq 0.973$). Pooled data from the replicates were mapped to non-overlapping genomic intervals (bins) to evaluate resolution. High-resolution Hi-C matrices achieved resolutions of 1 kb for the spotted seal, 2 kb for the goat, and 5 kb for the bottlenose dolphin and hog badger, enabling the detection of large-scale genomic features, such as A/B compartments, TADs, and enhancer-promoter interactions (Figure S9).

Marine mammals exhibited notable differences in chromatin compartment structure compared to their terrestrial relatives. In both the spotted seal and bottlenose dolphin, A compartments (open chromatin regions) were, on average, significantly shorter than B compartments (closed chromatin regions) (Table S15). Despite similar numbers of compartments and comparable distributions of genes within each compartment, the more compact A compartments in marine mammals suggest a denser gene distribution (Figure S10). TAD structures were largely conserved across the four genomes, with 2934, 2888, 2955, and 3090 TADs identified in the bottlenose dolphin, spotted seal, goat, and hog badger, respectively. Loop structures, representing significant interaction sites, were analyzed at 10-kb resolution using Fit-Hi-C.[32] This analysis revealed substantial variation in the number of intrachromosomal (*cis*) interaction sites, with 71,631, 258,867, 27,081, and 129,904 sites identified in the bottlenose dolphin, spotted seal, goat, and hog badger, respectively (Figure S11). Cross-species Hi-C comparisons identified convergent patterns in 3D genome organization between cetaceans and pinnipeds.

A total of 205 TADs were identified as uniquely conserved between the bottlenose dolphin and spotted seal, entirely enclosed within syntenic blocks and absent in their terrestrial relatives (Table S16). Within these conserved TADs, 670 genes were significantly enriched in GO terms related to neuronal development,

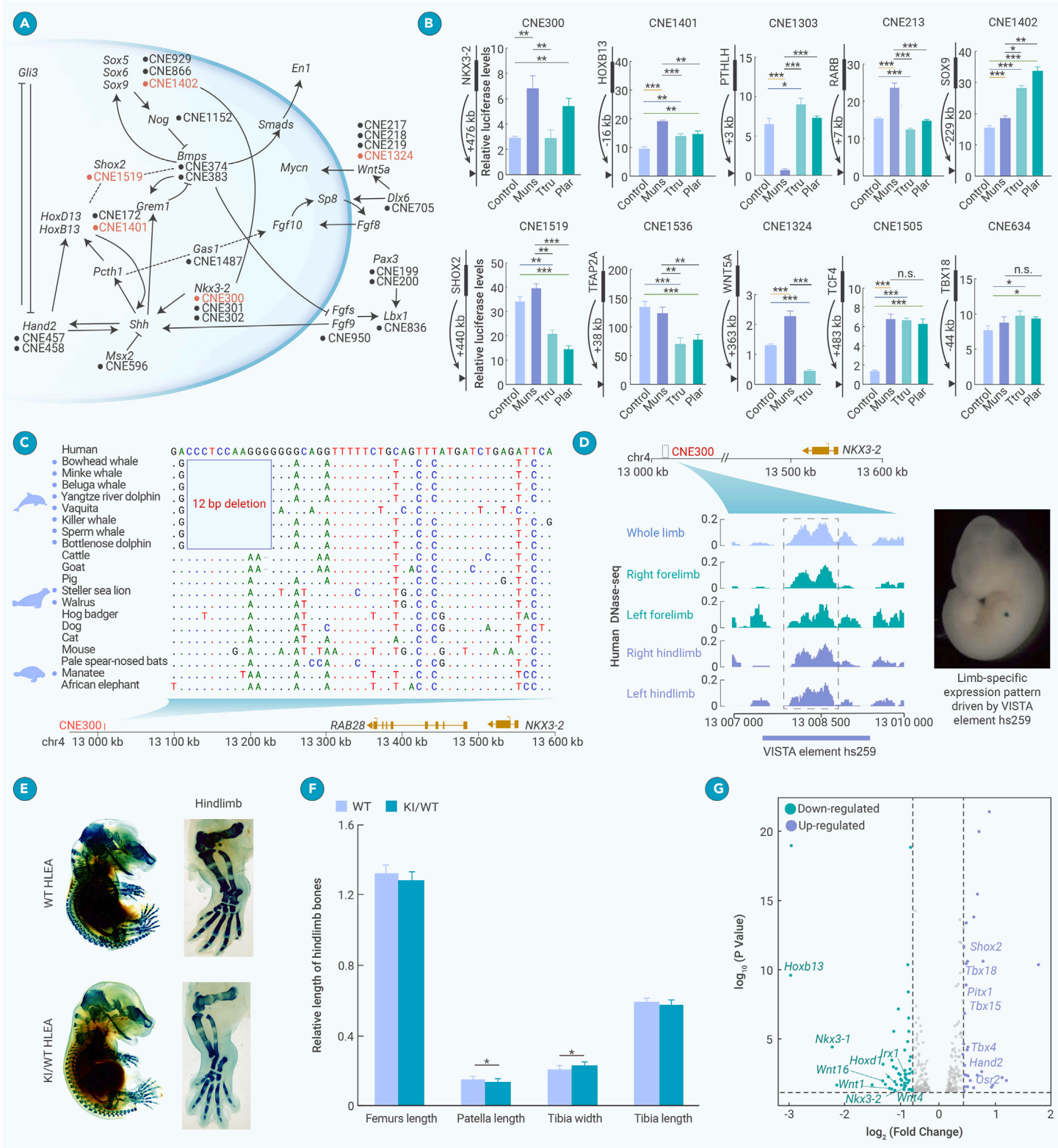


Figure 3. Adaptive genetic changes in regulatory elements associated with limb development (A) Marine mammal-specific CNEs broadly distributed in limb development patterning network, targeting genes involved in limb axis patterning, bud initiation, and outgrowth. Experimentally validated regulatory elements identified in this study are represented by red circles. (B) Functional differences in regulatory activity in marine-specific CNEs. *In vitro* reporter assays revealed that 8 out of 10 CNEs exhibited significant differences in reporter activity between marine mammals and orthologous mouse regions. Significance was tested using Student's t test. Mmus, mouse (*Mus musculus*); Plar, spotted seals (*Phoca largha*); Ttru: bottlenose dolphins (*T. truncatus*). (C) Sequence alignment of CNE300 showing cetacean-specific 12-bp deletion. Marine mammal lineages are shown in blue and terrestrial relatives are in black. (D) Predicted enhancer near *NKX3-2* identified through DNase-seq profiles and VISTA enhancer database in human developing limbs. (E) Skeletal morphology of HLEA heterozygous mice (KI/WT) compared to WT group at E15.5. Three individuals were analyzed per group. (F) Measurements of hindlimb bone components in HLEA KI/WT mice at E15.5, showing significantly decreased patella length and increased tibia width ($n = 3$) compared to WT mice. Error bars represent SEMs. Asterisk indicates significant difference at $p < 0.05$. (G) Volcano plot showing DEGs in HLEA KI/WT group versus WT group at E11.5. Cutoff values of log2fold change >1 and $p < 0.05$ were used to determine DEGs. Blue dots represent down-regulated genes, red dots represent up-regulated genes.

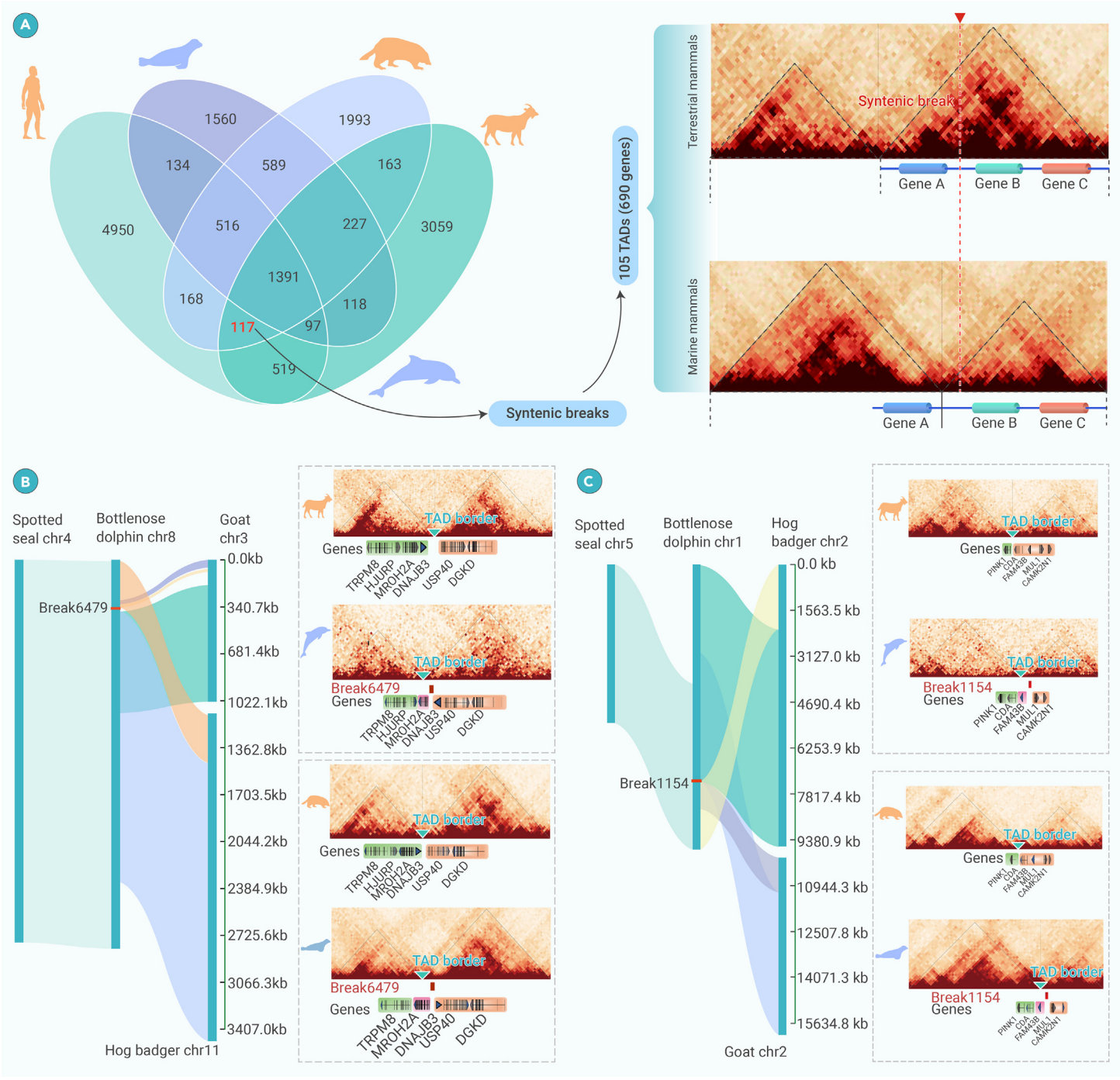


Figure 4. Chromosomal syntenic breaks and 3D genomic conformation (A) Genomic syntenic breaks (left) and their associated rearrangements in 3D chromatin organization in marine mammals (right). (B) Comparative genomics at break 6479. Insertion of break 6479 was observed in bottlenose dolphin and spotted seal genomes. Hi-C maps for bottlenose dolphin versus goat and spotted seal versus hog badger displaying TAD border (triangle), syntenic break (red square), and genes. Green and orange squares in Genes Track show different TADs. Genes located in the same TAD are shown in the same background. The magenta square represents TAD border. (C) Comparative genomics at break 1154. Hi-C maps for bottlenose dolphin versus goat and spotted seal versus hog badger displaying TAD borders (arrows), syntenic breaks (red squares), and genes. Green and orange squares in Genes Track show different TADs. Genes located in the same TAD show the same background. The magenta square represents TAD border.

lipoprotein biosynthetic process, and DNA repair (Table S17). Additionally, 117 syntenic blocks were distinctly disrupted in both the bottlenose dolphin and spotted seal, affecting 105 TADs and encompassing 690 annotated genes (Figure 4A; Table S18). Functional enrichment analyses of these genes revealed enrichment in GO terms and Kyoto Encyclopedia of Genes and Genomes pathways related to second messenger-mediated signaling, reproductive processes, ion transport, DNA strand elongation, base excision repair, vascular smooth muscle contraction, and temperature homeostasis (Tables S19 and S20).

A striking example of convergent TAD alterations was identified in lipid metabolism-related genes affected by syntenic break 6479 (Figure 4B; Table S20). In terrestrial mammals such as the goat and hog badger, *DNAJB3* and *MROH2A* were located within the same TAD. However, syntenic break 6479 disrupted this arrangement in the bottlenose dolphin and spotted seal (Figure 4B), repositioning *MROH2A* at the TAD boundary and relocating *DNAJB3* to an adjacent TAD. DNAJB3, a member of the HSP-40 protein family, plays a crucial role in modulating metabolic stress and maintaining glucose homeostasis.[33] *DNAJB3* deficiency has been shown to exacerbate diet-induced obesity and insulin resistance in mice,[33] emphasizing its importance in metabolic regulation. Further divergence in chromatin architecture was also observed for the *ASXL3* gene between marine mammals and their terrestrial relatives (Figure S12; Table S21). Notably, *NOL4* and *ASXL3* resided within the same TAD in goats but were located in different TADs in the bottlenose dolphin due to the insertion of syntenic break

12124. Similarly, in the hog badger, *ASXL3* spanned a TAD border, whereas the neighboring *CCDC178* gene was contained within a single TAD. In contrast, both loci were found within a single TAD in the spotted seal. TAD rearrangements can insulate promoters from enhancers, impacting their interactions and leading to gene dysregulation. Chromatin interaction analysis revealed significant differences in *ASXL3* activity across species. The bottlenose dolphin exhibited no detectable *cis*/*trans* interactions for *ASXL3*, compared to 4 in the goat, while the spotted seal showed only 1 interaction compared to 131 in the hog badger (Table S22). Functionally, *ASXL3* acts as a negative regulator of lipid accumulation by inhibiting the transcriptional activity of liver X receptor α, a nuclear receptor essential for cholesterol transport and lipogenesis.[34] In marine mammals, the syntenic breaks affecting *DNAJB3* and *ASXL3* likely perturb 3D genomic organization, reducing the ability to regulate lipid accumulation effectively. This disruption may contribute to enhanced insulin resistance and impaired lipogenesis inhibition, adaptations that align with the energy storage demands of thick blubber layers, a key feature of marine mammal physiology.

FAM43B, which suppresses prostate cancer cell proliferation,[35] exhibited distinct TAD positioning in marine and terrestrial mammals. In the goat and hog badger, *FAM43B* was located entirely within a TAD, whereas in the bottlenose dolphin and spotted seal, it was positioned at the boundary of the orthologous TAD, disrupted by syntenic break 1154 (Figure 4C). Chromatin interaction analyses revealed marked differences in the number of loops associated with *FAM43B*. The bottlenose dolphin exhibited 19 *cis*/*trans* interactions, while the spotted seal displayed 3. In contrast, no interactions were detected in the goat, and 38 were observed in the hog badger (Table S23). Chromatin loops can reposition distal genomic regions on chromosomes into close spatial proximity, thereby playing a crucial role in modulating gene expression.[36] Notably, bottlenose dolphin-specific chromatin interactions were identified in *FAM43B* (chr1) and *SMCHD1* (chr15) (Figure 4C). *SMCHD1* encodes a chromosomal architectural protein known to facilitate long-range chromatin interactions,[37] suggesting a potential regulatory link. We speculate that new chromatin interactions arising from changes in the bottlenose dolphin TADs may influence the transcriptional regulation of these genes.

SVs and their impact on nervous system development

SVs can induce large-scale perturbations of *cis*-regulatory regions, leading to significant alterations in gene expression and phenotypic outcomes. Based on genome-wide pairwise comparisons between marine mammals and their terrestrial relatives, marine species-specific SVs were identified by overlapping paired SV datasets. Validation was performed using both short-read and long-read sequencing for cetaceans and seals (see supplemental materials and methods). In total, 339 cetacean-specific SVs (68 deletions and 271 insertions) and 1054 pinniped-specific SVs (298 deletions and 756 insertions) were identified (Figures 5A and 5B; Table S24). Subsequent functional enrichment analysis revealed shared pathways and gene networks associated with head development and neuronal cell morphogenesis and development (Figure S13). For instance, six shared genes (*NUP153*, *ID4*, *DEK*, *CAP2*, *NHLRC1*, and *KIFL3A*) located at homologous genomic regions in the bottlenose dolphin (chr11: 13,000–16,000) and spotted seal (chr17: 135,000–140,000) (Table S25) were associated with brain development, cognitive function, and neurological diseases. In particular, *NUP153* was detected in three cetacean-specific SVs (ie, 589-bp, 689-bp, and 3 166-bp insertions) and two pinniped-specific SVs (ie, 95-bp and 225-bp insertions) (Figures 5C and 5D).

Hi-C domain predictions identified SV-associated genes located within TADs,[38] suggesting their susceptibility to regulatory changes. In total, 155 cetacean genes (30 TADs) and 414 pinniped genes (77 TADs) were identified (Table S26). Among these, *ID4*, a gene essential for maintaining neural stem cell quiescence,[39] was affected by 6 cetacean- and 11 pinniped-specific SVs within its TAD (Figures 5C and 5E). Among these, an SV near *ID4* in the bottlenose dolphin (6332-bp insertion) and spotted seal (641-bp deletion) was close (45 kb distance) to a known human forebrain enhancer (hs1175) in the VISTA Enhancer Browser[40] (Figure 5C). These SVs were also enriched with H3K27ac chromatin immunoprecipitation sequencing peaks, indicative of active regulatory regions in neocortical tissue.[41] These findings suggest that cetacean- and pinniped-specific SVs within the TAD encompassing ID4 may disrupt enhancer-promoter interactions, altering the expression and regulatory functions of this critical gene. Such SV-driven modifications in regulatory landscapes may underlie the unique neural adaptations observed in marine mammals, providing insights into the evolution of their advanced cognitive and neurological traits.

DISCUSSION

The transition from a terrestrial to an aquatic environment, known as secondary aquatic adaptation, marks a pivotal ecological shift in the macroevolution of terrestrial vertebrates. This shift, undertaken independently by three distinct marine mammal groups, underscores the profound ecological and morphological changes required to thrive in aquatic habitats. While it is well established that organisms frequently evolve analogous adaptations in response to similar environmental challenges, the genetic underpinnings of these convergent traits remain a topic of intense scientific debate. In the present study, molecular convergence was investigated at three levels: (1) shared coding genes with amino acid substitutions, (2) alterations in candidate regulatory elements, and (3) convergence in genomic architecture, specifically focusing on cetaceans and pinnipeds.

Blubber

To address the thermal challenges of aquatic environments, cetaceans, sirenians, and pinnipeds independently evolved a thick subcutaneous blubber layer, far exceeding the subcutaneous fat of terrestrial mammals.[42] Blubber serves multiple vital functions, including thermoregulation, buoyancy, energy storage, and locomotion. The metabolic processes involving the synthesis, storage, and catabolism of triacylglycerols, which are predominantly stored in adipocytes, are essential for life.[42]

A convergent amino acid substitution (P378L) in *APPL1* was identified across all three marine mammal lineages (Figure 2A). Subsequent functional analyses demonstrated that variation in this single amino acid was sufficient to alter its function, enhancing lipid accumulation in the 3T3-L1 murine preadipocyte cell line (Figures 2B–2D). Lipid metabolism-related adaptations were also observed in genome architecture. Syntenic breaks at loci 6479 and 12124 within the TAD containing *DNAJB3* and *ASXL3* disrupted 3D genomic organization (Figure 4B). These disruptions likely insulated promoters from enhancers, preventing their interactions and resulting in gene expression dysregulation.[43] Analogous alterations in both coding sequences and genome architecture across marine mammal species may have contributed to the evolution of blubber as an adaptive response to thermal challenges in aquatic environments.

Aquatic locomotion

Non-coding regulatory regions, subject to fewer pleiotropic constraints than coding regions,[22,23] may play a pivotal role in driving convergent phenotypic outcomes, such as limb reduction or complete loss.[5,44–46] Marine mammals have undergone remarkable morphological transformations to optimize aquatic locomotion, with the forelimbs and hindlimbs of pinnipeds evolving into flippers and the hindlimbs of cetaceans and manatees being lost completely.[1]

This study identified marine mammal-specific CNEs associated with accelerated evolution and limb morphology, exhibiting altered regulatory activity that likely contributed to aquatic adaptations (Figures 3A and 3B). Although these CNEs showed rapid evolutionary rates across marine mammal lineages, lineage-specific mutations were also apparent. These included a cetacean-specific 12-bp deletion in CNE300 and 4 deletions in the Tbx4 enhancer (HLEA), implying divergent evolutionary pathways (Figure 3C). Functional validation in KI mice carrying cetacean-specific deletions in HLEA corroborated its significant impact on limb phenotypes, underscoring its role in hindlimb regression (Figures 3E–3G). Despite these insights, the precise regulatory mechanisms through which these mutations influence limb development remain unclear, warranting further study. These findings pave the way for an in-depth exploration of conserved genetic regulatory networks that drive developmental processes and the molecular mechanisms underlying convergent evolution.

Brain and neuronal development

Marine mammals, particularly cetaceans and pinnipeds, exhibit exceptional levels of intelligence, sociability, and self-awareness.[47] Dolphins are second only to modern humans in terms of their significantly enlarged brains and high encephalization quotient, hypothesized to be key adaptations for navigating the complexities of social structures and communication within their communities.[47] In humans, genomic regions undergoing accelerated evolution are

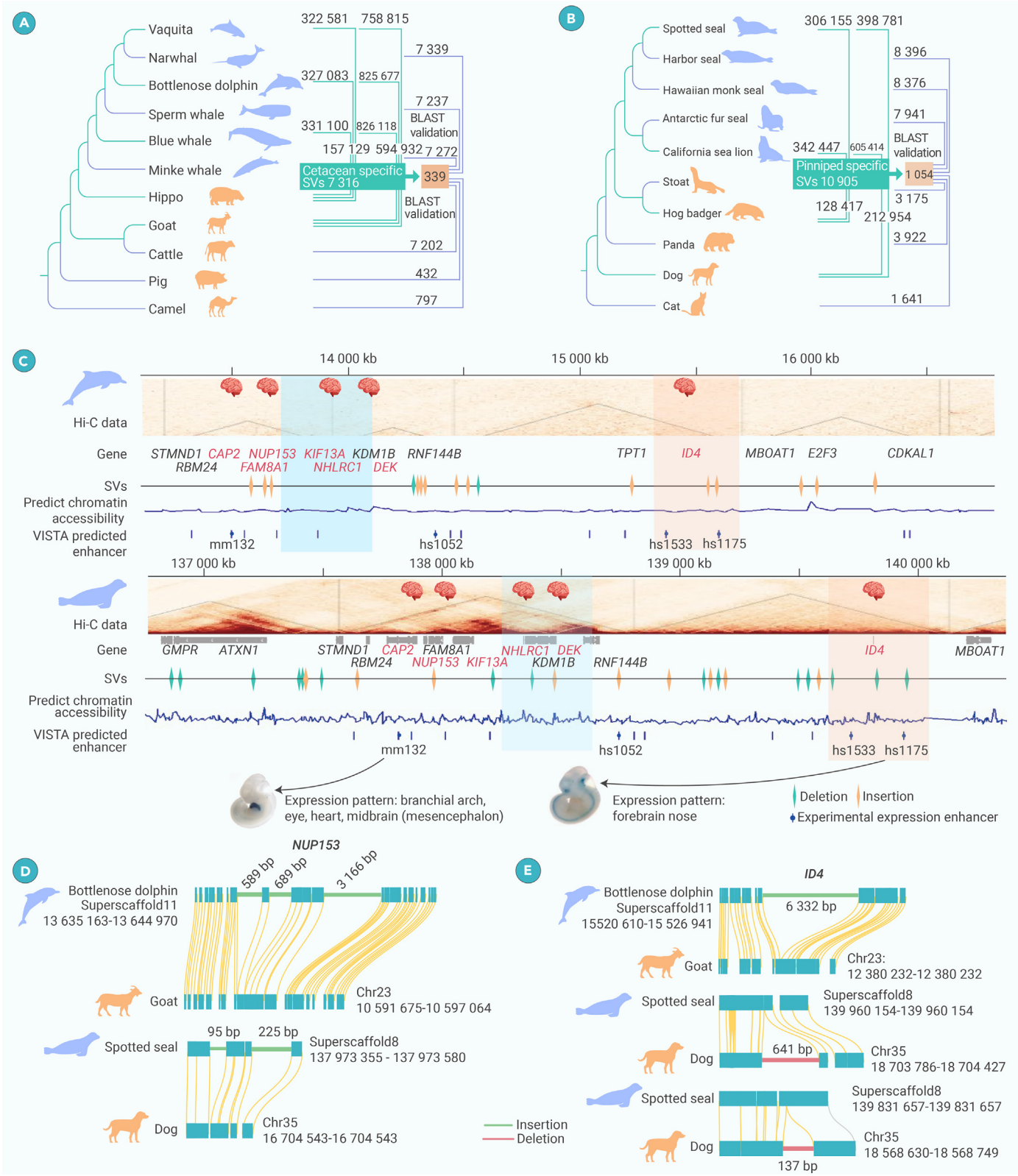


Figure 5. Marine mammal-specific SVs related to brain and neuronal development (A and B) Schematic representation of (A) cetacean- and (B) pinniped-specific SV calling. (C) Homologous genomic regions in cetaceans and pinnipeds susceptible to regulatory alterations by SVs, including VISTA-predicted enhancers. Enhancer activity at E11.5 in mouse embryos for mouse (mm132) and human (hs1175) enhancers, sourced from the VISTA database, shows tissue-specific expression patterns. Staining shows the expression pattern in tissues. (D and E) Miropeats diagrams of *NUP153*- and *ID4*-related SVs.

notably enriched in functions related to neurological development, which may underpin the evolutionary emergence of larger brain sizes and advanced cognitive traits, such as language.[48]

This study uncovered evidence of rapid evolution in CNEs regulating genes associated with neuronal development in marine mammalian lineages (Table S11). Furthermore, multiple SVs were identified within conserved TADs in both cetaceans and pinnipeds, which may influence the expression patterns of neuronal development-related genes, such as *NUP153* and *ID4* (Figures 5D and 5E). *NUP153* functions as an epigenetic determinant of adult neural stem cell maintenance and fate.[49] In mouse models of Alzheimer disease, reduced

NUP153 protein expression has been shown to impair hippocampal neural stem cell functionality.[49] Furthermore, dysregulated neurogenesis in the adult hippocampus has been identified as an early hallmark of Alzheimer disease, driving cognitive deterioration and memory dysfunction.[50] Studies have also shown that inhibition of *ID4* in mouse models of Alzheimer disease improves senescence-induced neuronal degeneration and promotes neurogenesis.[39] These findings suggest that evolutionary alterations in CNEs and SVs within marine mammal genomes may form the genetic basis for their advanced cognitive abilities and complex social behaviors. This convergent evolution of neurological traits underscores the role of genetic regulatory networks in shaping the remarkable intelligence and sociability of cetaceans and pinnipeds.

Cancer resistance

Marine mammals, especially cetaceans, have evolved larger body sizes and longer lifespans than their terrestrial counterparts. Conventional understanding suggests that these traits should elevate cancer risk, as a greater number of cells and more cell divisions over time increase the likelihood of accumulating mutations.[51] However, empirical evidence defies this expectation, revealing no correlation between body size, lifespan, and cancer incidence across species—often referred to as Peto's paradox.[51] Cetaceans, which include the 10 longest-lived mammals in the world apart from humans,[52] exhibit cancer incidence rates as low as 0.7%–2.0%,[53] markedly lower than the 11%–25% observed in humans.[54] This striking disparity underscores the evolution of highly efficient anticancer mechanisms in marine mammals, which likely contribute to their exceptional longevity.

In this study, 27 cancer-related genes exhibiting convergent evolution were identified across the marine mammal lineages. Among these, a radical amino acid substitution (E71G) in *NEIL1* was shown to significantly reduce the proliferation of human cervical cancer cells, suggesting a potential role in enhancing cancer resistance and promoting longevity (Figure 2F). Changes in the cancer-related gene *FAM43B* were also observed, attributable to a TAD disruption induced by syntenic break 1154 (Figure 4C). These modifications in chromatin structure may disrupt enhancer-promoter interactions, altering transcriptional regulation and contributing to the evolution of anticancer adaptations. The evolutionary strategies that marine mammals employ to mitigate cancer risk offer a unique perspective on the mechanisms underlying cellular resilience. Deciphering these natural anticancer defenses in large, long-lived species may yield transformative insights into combating cancer and enhancing longevity in other organisms, including humans.

DATA AND CODE AVAILABILITY

All whole-genome sequence data reported in this paper were deposited in the Genome Warehouse in the National Genomics Data Center (CNCB-NGDC Members and Partners 2022), Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under accession numbers GWHBJYT00000000, GWHBJYR00000000, and GWHBJYS00000000 (publicly accessible at https://ngdc.cncb.ac.cn/gwh). This paper does not report any original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

REFERENCES

1. Berta, A., Sumich, J.L. and Kovacs, K.M. (2005). Marine Mammals: Evolutionary Biology (Elsevier Press), pp. 1–2. DOI:https://doi.org/10.1016/C2011-0-07338-6.
2. Foote, A.D., Liu, Y., Thomas, G.W.C. et al. (2015). Convergent evolution of the genomes of marine mammals. *Nat. Genet.* **47**:272–275.
3. He, Z., Xu, S. and Shi, S. (2020). Adaptive convergence at the genomic level-prevalent, uncommon or very rare? *Natl. Sci. Rev.* **7**:947–951.
4. Thomas, G.W.C., Hahn, M.W. and Hahn, Y. (2017). The effects of increasing the number of Taxa on inferences of molecular convergence. *Genome Biol. Evol.* **9**:213–221.
5. Sackton, T.B., Grayson, P., Cloutier, A. et al. (2019). Convergent regulatory evolution and loss of flight in paleognathous birds. *Science* **364**:74–78.
6. Roscito, J.G., Sameith, K., Kirilenko, B.M. et al. (2022). Convergent and lineage-specific genomic differences in limb regulatory elements in limbless reptile lineages. *Cell Rep.* **38**:110280–110295.
7. Roscito, J.G., Sameith, K., Parra, G. et al. (2018). Phenotype loss is associated with widespread divergence of the gene regulatory landscape in evolution. *Nat. Commun.* **9**:4737–4752.
8. Partha, R., Chauhan, B.K., Ferreira, Z. et al. (2017). Subterranean mammals show convergent regression in ocular genes and enhancers, along with adaptation to tunneling. *Elife* **6**:e25884–e25910.
9. Polychronopoulos, D., King, J.W.D., Nash, A.J. et al. (2017). Conserved non-coding elements: developmental gene regulation meets genome organization. *Nucleic Acids Res.* **45**:12611–12624.
10. Yuan, Y., Zhang, Y., Zhang, P. et al. (2021). Comparative genomics provides insights into the aquatic adaptations of mammals. *Proc. Natl. Acad. Sci. USA* **118**:e2106080118.
11. Cicconardi, F., Lewis, J.J., Martin, S.H. et al. (2021). Chromosome fusion affects genetic diversity and evolutionary turnover of functional loci but consistently depends on chromosome size. *Mol. Biol. Evol.* **38**:4449–4462.
12. Jain, Y., Chandradoss, K.R., AV, A. et al. (2021). Convergent evolution of a genomic rearrangement may explain cancer resistance in hystrico- and sciuromorpha rodents. *NPJ Aging Mech. Dis.* **7**:20–35.
13. Li, X., Wang, J., Yu, Y. et al. (2023). Genomic rearrangements and evolutionary changes in 3D chromatin topologies in the cotton tribe (Gossypieae). *BMC Biol.* **21**:56–73.
14. Mohr, D.W., Gaughran, S.J., Paschall, J. et al. (2022). A chromosome-length assembly of the Hawaiian Monk Seal (*Neomonachus schauinslandi*): a history of "genetic purging" and genomic stability. *Genes* **13**:1270–1286.
15. Simão, F.A., Waterhouse, R.M., Ioannidis, P. et al. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**:3210–3212.
16. Kumar, S., Stecher, G., Suleski, M. et al. (2017). TimeTree: a resource for timelines, timetrees, and divergence times. *Mol. Biol. Evol.* **34**:1812–1819.
17. Scornavacca, C., Belkhir, K., Lopez, J. et al. (2019). OrthoMaM v10: scaling-up orthologous coding sequence and exon alignments with more than one hundred mammalian genomes. *Mol. Biol. Evol.* **36**:861–862.
18. Park, M., Wu, D., Park, T. et al. (2013). APPL1 transgenic mice are protected from high-fat diet-induced cardiac dysfunction. *Am. J. Physiol. Endocrinol. Metab.* **305**:E795–E804.
19. Saeed, S., Bonnefond, A., Tamanini, F. et al. (2018). Loss-of-function mutations in ADCY3 cause monogenic severe obesity. *Nat. Genet.* **50**:175–179.
20. Wen, Z., Tang, Z., Li, M. et al. (2020). APPL1 knockdown blocks adipogenic differentiation and promotes adipocyte lipolysis. *Mol. Cell. Endocrinol.* **506**:110755–110764.
21. Vartanian, V., Lowell, B., Minko, I.G. et al. (2006). The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase. *Proc. Natl. Acad. Sci. USA* **103**:1864–1869.
22. Carroll, S.B., Prud'homme, B. and Gompel, N. (2008). Regulating evolution. *Sci. Am.* **298**:60–67.
23. Chan, Y.F., Marks, M.E., Jones, F.C. et al. (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* **327**:302–305.
24. Hecker, N. and Hiller, M. (2020). A genome alignment of 120 mammals highlights ultraconserved element variability and placenta-associated enhancers. *GigaScience* **9**:giz159–giz169.
25. Ming, Z., Vining, B., Bagheri-Fam, S. et al. (2022). SOX9 in organogenesis: shared and unique transcriptional functions. *Cell. Mol. Life Sci.* **79**:522–547.
26. Yao, B., Wang, Q., Liu, C.F. et al. (2015). The SOX9 upstream region prone to chromosomal aberrations causing campomelic dysplasia contains multiple cartilage enhancers. *Nucleic Acids Res.* **43**:5394–5408.
27. Thewissen, J.G.M., Cohn, M.J., Stevens, L.S. et al. (2006). Developmental basis for hind-limb loss in dolphins and origin of the cetacean bodyplan. *Proc. Natl. Acad. Sci. USA* **103**:8414–8418.
28. Liang, N., Deme, L., Kong, Q. et al. (2022). Divergence of Tbx4 hindlimb enhancer HLEA underlies the hindlimb loss during cetacean evolution. *Genomics* **114**:110292–110299.
29. Menke, D.B., Guenther, C. and Kingsley, D.M. (2008). Dual hindlimb control elements in the Tbx4 gene and region-specific control of bone size in vertebrate limbs. *Development* **135**:2543–2553.
30. Swank, S., Sanger, T.J. and Stuart, Y.E. (2021). (Non)Parallel developmental mechanisms in vertebrate appendage reduction and loss. *Ecol. Evol.* **11**:15484–15497.
31. Bickhart, D.M., Rosen, B.D., Koren, S. et al. (2017). Single-molecule sequencing and chromatin conformation capture enable *de novo* reference assembly of the domestic goat genome. *Nat. Genet.* **49**:643–650.
32. Ay, F., Bailey, T.L. and Noble, W.S. (2014). Statistical confidence estimation for Hi-C data reveals regulatory chromatin contacts. *Genome Res.* **24**:999–1011.
33. Nejat, S., Scoggin, S., Menikdiwela, K. et al. (2021). DNAJB3 deficiency exacerbates diet-Induced obesity and insulin resistance. *Curr. Dev. Nutr.* **5**:1235.
34. Shin, N., Lee, Y.K., Park, U.H. et al. (2014). Repression of LXRalpha by a novel member of additional sex comb-like family, ASXL3. *Biochem. Biophys. Res. Commun.* **454**:479–485.
35. Liang, T., Ye, X., Liu, Y. et al. (2018). FAM46B inhibits cell proliferation and cell cycle progression in prostate cancer through ubiquitination of beta-catenin. *Exp. Mol. Med.* **50**:1–12.
36. Dekker, J. and Misteli, T. (2015). Long-range chromatin interactions. *Cold Spring Harbor Perspect. Biol.* **7**:a019356.
37. Jansz, N., Keniry, A., Trussart, M. et al. (2018). Smchd1 regulates long-range chromatin interactions on the inactive X chromosome and at Hox clusters. *Nat. Struct. Mol. Biol.* **25**:766–777.
38. Luo, X., Liu, Y., Dang, D. et al. (2021). 3D Genome of macaque fetal brain reveals evolutionary innovations during primate corticogenesis. *Cell* **184**:723–740.e21.
39. Zhang, R., Boareto, M., Engler, A. et al. (2019). Id4 Downstream of Notch2 maintains neural stem cell quiescence in the adult hippocampus. *Cell Rep.* **28**:1485–1498.e6.
40. Visel, A., Minovitsky, S., Dubchak, I. et al. (2007). VISTA Enhancer Browser–a database of tissue-specific human enhancers. *Nucleic Acids Res.* **35**:D88–D92.
41. Rubin, A.N., Malik, R., Cho, K.K.A. et al. (2020). Regulatory elements inserted into aavs confer preferential activity in cortical interneurons. *Eneuro* **7**:1–20.

42. Wursig, B. and Perrin, W.F. (2009). Encyclopedia of Marine Mammals (Academic Press), pp. 115–119. DOI:https://doi.org/10.1016/B978-0-12-373553-9.X0001-6.
43. Li, L., Barth, N.K.H., Pilarsky, C. et al. (2019). Cancer is associated with alterations in the three-dimensional organization of the genome. *Cancers* **11**:1886–1907.
44. Peng, C., Wu, D.D., Ren, J.L. et al. (2023). Large-scale snake genome analyses provide insights into vertebrate development. *Cell* **186**:2959–2976.e22.
45. Wang, Z., Peng, C., Wu, W. et al. (2023). Developmental regulation of conserved non-coding element evolution provides insights into limb loss in squamates. *Sci. China Life Sci.* **66**:2399–2414.
46. Kvon, E.Z., Kamneva, O.K., Melo, U.S. et al. (2016). Progressive loss of function in a limb enhancer during snake evolution. *Cell* **167**:633–642.e11.
47. Marino, L. (2020). Large brains in small tanks: intelligence and social complexity as an ethical issue for captive dolphins and whales. In Neuroethics and Nonhuman Animals (Springer Press), pp. 177–189. DOI:https://doi.org/10.1007/978-3-030-31011-0_10.
48. Won, H., Huang, J., Opland, C.K. et al. (2019). Human evolved regulatory elements modulate genes involved in cortical expansion and neurodevelopmental disease susceptibility. *Nat. Commun.* **10**:2396–2407.
49. Leone, L., Colussi, C., Gironi, K. et al. (2019). Altered Nup153 expression impairs the function of cultured hippocampal neural stem cells isolated from a mouse model of Alzheimer's Disease. *Mol. Neurobiol.* **56**:5934–5949.
50. Babcock, K.R., Page, J.S., Fallon, J.R. et al. (2021). Adult hippocampal neurogenesis in Aging and Alzheimer's Disease. *Stem Cell Rep.* **16**:681–693.
51. Caulin, A.F. and Maley, C.C. (2011). Peto's Paradox: evolution's prescription for cancer prevention. *Trends Ecol. Evol.* **26**:175–182.
52. Tacutu, R., Thornton, D., Johnson, E. et al. (2018). Human Ageing Genomic Resources: new and updated databases. *Nucleic Acids Res.* **46**:D1083–D1090.
53. Poirier, M.C. (2021). DNA damage in cetaceans: A mini review. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **870–871**:503392.
54. Abegglen, L.M., Caulin, A.F., Chan, A. et al. (2015). Potential mechanisms for cancer resistance in elephants and comparative cellular response to dna damage in humans. *JAMA* **314**:1850–1860.

ACKNOWLEDGMENTS

This work was supported by the National Key Programme of Research and Development, Ministry of Science and Technology (2022YFF1301600) to G.Y.; the Key Project of the National Natural Science Foundation of China (32030011) to G.Y.; the National Natural Science Foundation of China (32070409, 32270453, 32270442, 32070408, and 32270441) to S.X., W.R., L.S., and R.T.; the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Qinglan Project of Jiangsu Province to S.X.; the Specially-Appointed Professor Program of Jiangsu Province to I.S.; the Jiangsu Foreign Expert Bureau to I.S.; the Jiangsu Provincial Department of Technology (JSSCTD202142) to I.S.; and the PI Project of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2021GD0805) to G.Y. The funders had no role in study design, data collection, and analysis; decision to publish; or preparation of the manuscript. We are very grateful to Prof. Qiang Qiu for assistance in the bioinformatics analysis. We thank Xin Huang for help in the bioinformatics analysis. Many thanks also to Liuming Gao and Xiang Zhu (Nanjing Underwater World) and Dong Wu and Gang Qian (Ningbo Shenfeng Ocean World Company) for help during sampling. We also thank Lin Liu and Wenchao Nie for Hi-C library construction and sequencing and Wuhan Frasergen Bioinformatics Company for support.

AUTHOR CONTRIBUTIONS

G.Y., S.X., and L.S. conceived the project and designed the research. G.Y., L.S., S.X., and R.T. collected the materials. L.S., R.T., Z.Y., and D.S. performed the genome assembly, annotation, and evolutionary analyses. M.Z., L.S., and Z.Z. implemented the *in vitro* experiments. N.L. conducted the *in vivo* experiments. G.Y., W.R., D.Y., S.C., and I.S. participated in discussions and provided suggestions. S.X., L.S., R.T., Z.Y., and D.S. wrote the manuscript, with input from all co-authors. All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

It can be found online at https://doi.org/10.1016/j.xinn.2025.100798.