



RESEARCH ARTICLE

Evolutionary Biology and Gene Editing of Cat Allergen, Fel d 1

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Abstract

Allergy to domestic cat affects up to 15% of the population, and sensitization to cat allergen is associated with asthma. Despite the pervasiveness of cat allergic disease, current treatments have limited impact. Here, we present a bioinformatics analysis of the major cat allergen, Fel d 1, and demonstrate proof of principle for CRISPR gene editing of the allergen. Sequence and structural analyses of Fel d 1 from 50 domestic cats identified conserved coding regions in genes *CH1* and *CH2* suitable for CRISPR editing. Comparative analyses of Fel d 1 and orthologous sequences from eight exotic felid species determined relatively low-sequence identities for *CH1* and *CH2*, and implied that the allergen may be nonessential for cats, given the apparent lack of evolutionary conservation. *In vitro* knockouts of domestic cat Fel d 1 using CRISPR-Cas9 yielded editing efficiencies of up to 55% and found no evidence of editing at predicted potential off-target sites. Taken together, our data indicate that Fel d 1 is both a rational and viable candidate for gene deletion, which may profoundly benefit cat allergy sufferers by removing the major allergen at the source.

Introduction

Domestic cat (*Felis catus*) is the most common source of mammalian allergen, with allergy to cat affecting up to 15% of adults and children and producing symptoms that range from rhinoconjunctivitis to severe asthma.^{1–3} Recent data from the U.S. National Health and Nutrition Examination Survey attributed more than 500,000 asthma attacks per year and ~350,000 emergency care visits to patients who were sensitized and exposed to cat allergen.⁴ Despite the prevalence and potential severity of cat allergic disease, current treatments merely address the allergic symptoms (e.g., antihistamines) or have demonstrated inconsistent results (e.g., cat allergen immunotherapy^{5,6}).

The major cat allergen, Fel d 1, is a 35 kD tetrameric protein composed of two heterodimers, each of which consists of two chains: chain 1 (70 aa, 8 kD protein) and chain 2 (92 aa, 10 kD).^{7,8} The genes *CH1* and *CH2* (human gene nomenclature used here for cat), encoding chains 1 and 2, respectively, are located in a genomic span of <10,000 bp (chromosome E2, base pairs ~18,487,507–18,496,763;

NCBI assembly *Felis_catus*_8.0). The crystal structure of recombinant Fel d 1 shows that the protein binds Ca²⁺ ions and has two amphipathic internal cavities that may bind steroid ligands.⁹ Fel d 1 is a secretoglobin¹⁰ produced by cat salivary,¹¹ sebaceous,¹² perianal,¹³ and lachrymal¹¹ glands. Natural levels of the allergen vary significantly between cats (>100-fold) and even within the same cat.¹⁴

Fel d 1 causes IgE antibody-mediated sensitization in ~95% of cat allergic patients and accounts for 60–90% of the anti-cat IgE.^{15–19} High-level exposure to Fel d 1 drives modified Th2 immune responses that elicit IgG4 antibody production in allergic and nonallergic individuals.²⁰ Though several other cat allergens have been identified (e.g., Fel d 4), they are of less allergenic and clinical significance.^{21,22}

While the biologic function of Fel d 1 is unknown, studies suggest that the protein may play a role in chemical communication, epithelium defense, or immune regulation.^{7,23–26} To date, there have been no documented knockouts of Fel d 1 in cats. The Fel d 1 genes are not expressed in species beyond the family *Felidae*, though

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homologous sequences with ~25–50% identity have been identified in rabbit (uteroglobin protein⁷), human (Clara cell 10 kD secretory protein⁷), mouse (androgen-binding protein²⁷), and the slow loris primate (brachial gland secretion protein²⁴).

Genome engineering presents a unique opportunity for the unequivocal deletion of Fel d 1 from cat cells and tissues and may provide the critical step in establishing the definitive function of the allergen. CRISPR in particular offers considerable improvements in throughput, target specificity, editing efficiency, and precision compared to prior technologies.²⁸ Additionally, CRISPR has demonstrated promise in therapeutic applications (e.g., sickle cell disease and β -thalassemia²⁹) and offers profound clinical potential. Given the advantages of CRISPR gene editing and a specific genomic target in Fel d 1, CRISPR is a practical approach for tackling cat allergic disease, which may greatly improve the lives of cat-allergic individuals.

Here, we report a structural bioinformatics analysis of Fel d 1 to optimize single-guide RNA (sgRNA) site selection for CRISPR editing of the allergen. This approach has enabled the evolutionary relationships between Fel d 1 and exotic cat orthologs to be established and provides novel insight into the biologic function of the allergen. The results indicate that Fel d 1 is amenable to gene editing using CRISPR and provide the first step in creating Fel d 1 knockout cats.

Methods

Comparative analyses of domestic cat Fel d 1 and exotic cat orthologs

Domestic cat DNA extraction and Fel d 1 sequence analysis. Discarded tissue samples (feline testes, ovaries, uteri) from the spay or neuter procedures of 50 domestic cats (98% domestic short- or long-haired cats of mixed ancestry) were generously provided for the study (Charlottesville Albemarle SPCA).

Genomic DNA was extracted from the samples (DNeasy[®] Blood & Tissue Kit; Qiagen), and *CH1* and *CH2* were polymerase chain reaction (PCR) amplified (Supplementary Table S1), purified (QIAquick[®] PCR Purification Kit; Qiagen), Sanger sequenced (Eurofins Genomics), and translated (ExPASy). Pairwise and multisequence alignments of the Fel d 1 DNA or protein sequences with the GenBank references (DNA: M74952.1 and M77341.1; protein: AAC37318.1 and AAC41616.1) were performed, and percent identity was determined (CLUSTAL Omega, Geneious Prime).

Sequence analysis of exotic cat Fel d 1 orthologs. The DNA reference sequences for full-length (including introns) Fel d 1 chain 1 (NCBI accession X62477) and

chain 2 (NCBI accession X62478) were used to perform a basic local alignment search (BLAST) of 24 exotic (nondomestic) cat genomes available via the NCBI sequence read archive database (Supplementary Table S2). Eight big or wild felid species were analyzed ($n=3$ cats per exotic species), including Chinese mountain cat (*Felis silvestris bieti*, a potential subspecies of *Felis silvestris*^{30–33}), black-footed cat (*Felis nigripes*), leopard cat (*Prionailurus bengalensis*), fishing cat (*Prionailurus viverrinus*), cougar (*Puma concolor*), Iberian lynx (*Lynx pardinus*), Bengal tiger (*Panthera tigris tigris*), and African lion (*Panthera leo*).

The resulting whole-genome sequencing fragments were assembled to the Fel d 1 references to yield DNA consensus sequences, which were then translated (Geneious Prime). Pairwise and multisequence alignments of the exotic cat DNA or protein orthologs with the GenBank Fel d 1 references were performed, and percent identity was determined (CLUSTAL Omega, Geneious Prime). Unrooted, neighbor-joining phylogenetic trees of Fel d 1 chains 1 and 2 were built from multisequence alignments of the 50 domestic and 24 exotic cat sequences (Geneious Prime).

Selective pressure analysis of Fel d 1 orthologous sequences.

Selective pressure was estimated by comparing the GenBank DNA references for Fel d 1 *CH1* or *CH2* with the exotic cat DNA orthologs (one representative cat per exotic species). The numbers of synonymous sites (S), synonymous substitutions (DS), nonsynonymous sites (N), and nonsynonymous substitutions (DN) were determined, and the synonymous and nonsynonymous substitution rates (dS and dN, respectively) were calculated.³⁴ The ratio of the nonsynonymous and synonymous substitution rates ($\omega = dN/dS$) was determined for each exotic species (Supplementary Table S3). If no synonymous substitutions were present, the dN/dS ratio was estimated with the alternative: $\omega = dN/((DS + 1)/S)$.

Gene editing of Fel d 1 using CRISPR-Cas9

CRISPR sgRNA design. Conserved regions of domestic cat *CH1* and *CH2* were identified by sequence analysis (Geneious Prime). CRISPR sgRNAs with sequences complementary to conserved coding regions of Fel d 1 and adjacent to CRISPR-Cas9 specific protospacer adjacent motif (PAM) sequences (“NGG”) and with few predicted potential off-target cleavage sites were designed (CRISPOR³⁵). Ten Fel d 1-specific sgRNAs were selected and synthesized (Invitrogen; Supplementary Table S4).

Feline cell culture. Immortalized feline kidney epithelial cells (Crandell–Rees Feline Kidney [CRFK]; ATCC CCL-94) were grown in Eagle’s Minimum Essential Medium

(EMEM) supplemented with 10% horse serum (Thermo Fisher Scientific) and 1% penicillin-streptomycin (ATCC). The cells were maintained at 37°C with 5% CO₂ and passaged every 2–3 days using 0.25% Trypsin-EDTA (ATCC).

CRISPR transfection of feline cells. Feline CRFK cells were seeded in antibiotic-free, serum-rich EMEM at 250,000 cells/well on six-well plates ~24 h prior to transfection. The *CHI*- or *CH2*-specific sgRNAs and Cas9 nuclease (TrueCut Cas9 Protein v2; Invitrogen) were delivered to the cells in a 1:1 molar ratio by lipid-based transfection (Lipofectamine CRISPRMAX; Invitrogen), and the transfected cells were incubated at 37°C with 5% CO₂ for 48 h. The editing efficiencies of each of the 10 Fel d 1 sgRNAs were evaluated individually, with three biological replicates per sgRNA. A noncoding sgRNA was used as a negative control, while sgRNAs targeted to feline hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) served as positive transfection and CRISPR controls (Invitrogen; Supplementary Table S5).

Enzymatic detection of CRISPR edited Fel d 1. Control and edited CRFK cells were lysed 48 h post transfection (Genomic Cleavage Detection Kit; GeneArt). Partial *CHI* or *CH2* sequences were PCR amplified around the predicted CRISPR cleavage sites (Supplementary Table S6). The resulting DNA fragments were denatured and randomly re-annealed to form heterogeneous DNA duplexes, which were incubated with T7 endonuclease 1 (T7E1, Genomic Cleavage Detection Kit, GeneArt; Alt-R Genome Editing Detection Kit, IDT) and run on a 2% agarose gel at 100 V for 1 h.

The gel was imaged (DigiDoc-It) and analyzed (ImageJ) to estimate editing efficiency from the gel band intensities given editing efficiency = $1 - [(1 - \text{fraction cleaved})^{1/2}]$, where fraction cleaved = sum of cleaved band intensities / (sum of cleaved and parent band intensities).

Sequence decomposition of CRISPR edited Fel d 1. Control and CRISPR edited CRFK cells were lysed 48 h post transfection (Genomic Cleavage Detection Kit; GeneArt). Partial *CHI* or *CH2* sequences were PCR amplified around the predicted CRISPR cleavage sites (Supplementary Table S6), and the resulting DNA fragments were purified (QIAquick® PCR Purification Kit; Qiagen) and Sanger sequenced (Eurofins Genomics).

The control and edited DNA chromatogram traces were uploaded to the Tracking of Indels by Decomposition (TIDE³⁶) and Inference of CRISPR Edits (ICE; Synthego) bioinformatics platforms. TIDE and ICE determined all possible edits for the specified control

trace, and used regression analysis to estimate sgRNA editing efficiency based on the relative abundance of each indel (insertion/deletion) in the mixed DNA trace of the CRISPR edited sample.³⁷

CRISPR off-target identification and detection. Potential sites of off-target CRISPR editing were identified for sgRNAs C1G1 and C2G1 (CRISPOR³⁵). Four predicted potential off-target sites (C1G1: *ENSCAG00000030374-AQP4* [intergenic], *OSMR-LIFR* [intergenic]; C2G1: *KIZ* [intron], *TEX264* [exon]) with three nucleotide mismatches from the C1G1 or C2G1 target sequences were evaluated. Control and edited CRFK DNA was PCR amplified around the predicted off-targets (Supplementary Table S7). Editing efficiency of C1G1 or C2G1 at the off-target sites was evaluated by sequence decomposition (TIDE, ICE) and T7E1 enzymatic detection.

Results

Sequence and structural analysis of domestic cat Fel d 1

CHI and *CH2* were sequenced from 50 domestic cats to identify protein variants or amino acid substitutions, as well as conserved DNA target regions for CRISPR sgRNA design. From the domestic cat protein sequences, 37 unique amino acid substitutions were identified, including 18 novel, natural Fel d 1 variants resulting from seven single nucleotide polymorphisms (SNPs), four multiple nucleotide polymorphisms (MNP), or seven rare mutations (polymorphisms observed in a single cat; Fig. 1A and Supplementary Table S8).

The domestic cat variants were mapped onto the tetrameric crystal structure of Fel d 1 (PDB 2EJN⁹) and were found to be primarily concentrated at the interface of the two dimers (Fig. 1B). Several patterns of correlated Fel d 1 variants were revealed, and notably, 42% of the domestic cats encoded a noncanonical isoform, CH2S,⁸ or a similar novel variant at residues Thr82-Asp89 (Fig. 1A and B and Supplementary Table S8). The domestic cat Fel d 1 variants are highlighted in an accompanying interactive visualization (www.feld1viz.com).

Multiple conserved coding regions in the Fel d 1 genes with suitable target sequences for CRISPR editing were revealed. Fel d 1 sequence fragments with more than 20 well-conserved (>98% identity), consecutive nucleotides adjacent to a CRISPR-Cas9 PAM sequence (“NGG”) were deemed appropriate targets for CRISPR editing. Given these criteria, we identified two conserved CRISPR target regions in *CHI* (Gly19-Tyr50, Ala52-Ser81) and three regions in *CH2* (Met1-Gln14, Leu16-Tyr28, Ser45-Leu71; Fig. 1A and C). These conserved sequences guided the design of 10 Fel d 1-specific CRISPR sgRNAs (Supplementary Table S4).

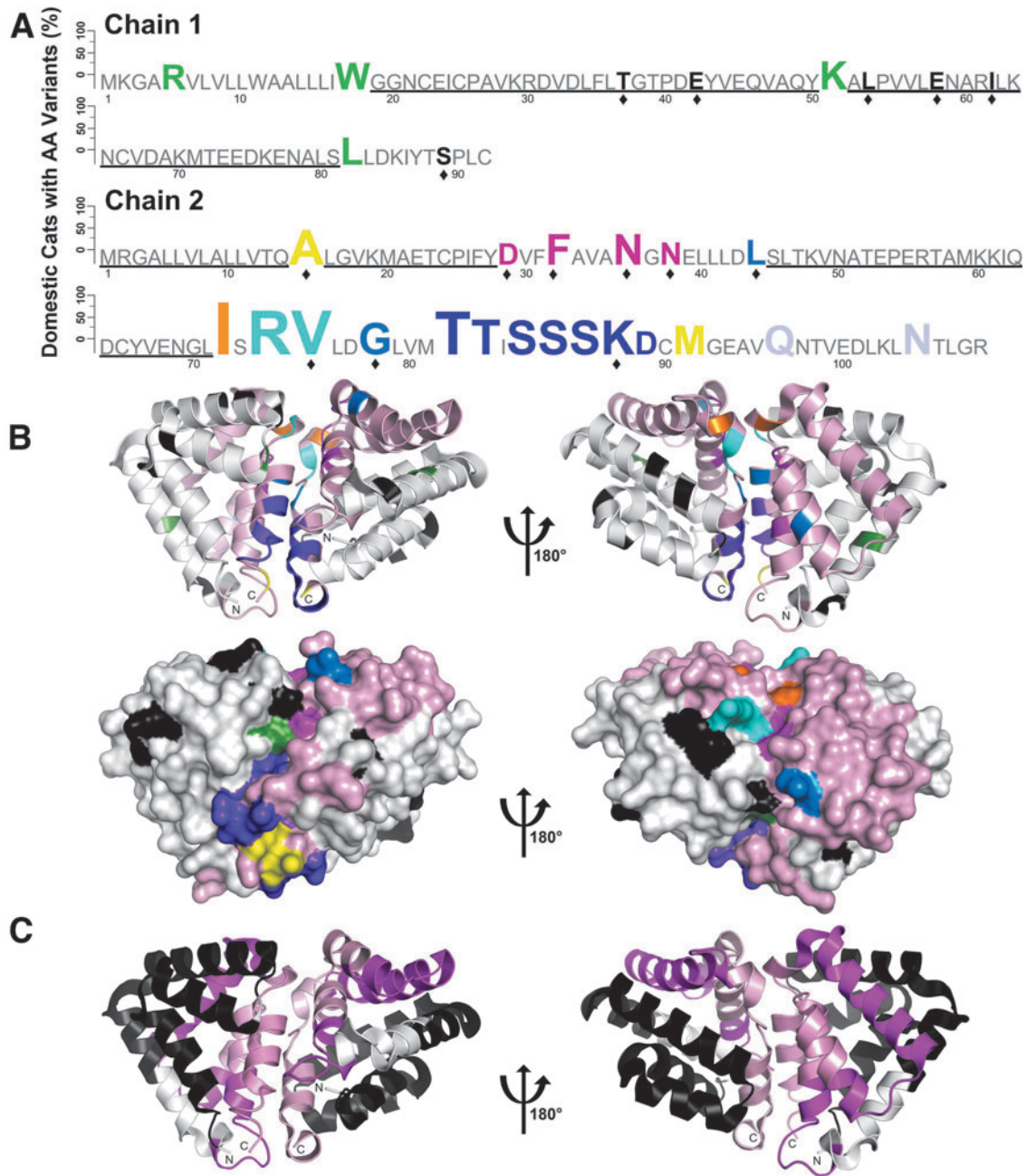


FIG. 1. Comprehensive sequence and structural analyses of domestic cat Fel d 1. Fel d 1 chains 1 and 2 were sequenced from 50 domestic cats. **(A)** Amino acid sequences of Fel d 1 chains 1 and 2 (GenBank reference sequences AAC37318.1 and AAC41616.1, respectively). The percentage of domestic cats with sequence variants at a given amino acid residue (font size) and the patterns of correlated variants (font color) were evaluated. Several novel Fel d 1 amino acid variants (♦) as well as conserved target regions suitable for editing with CRISPR-Cas9 (underlined) were identified. **(B)** Ribbon (top) and surface (bottom) structures (PDB 2EJN⁹) of Fel d 1. Each molecule is a heterodimer of chain 1 (white) and chain 2 (light pink), which form a tetramer in the natural allergen. Correlated Fel d 1 variants (grouped by color) that were identified from the 50 domestic cats were found to accumulate along the midline of the tetrameric structure at the interface of the two Fel d 1 dimers. **(C)** Ribbon structure of Fel d 1 chain 1 (white) and chain 2 (light pink) showing conserved regions of the genes with appropriate target sequences for genome editing with CRISPR-Cas9 indicated (chain 1: black; chain 2: magenta).

Multisequence alignments of the domestic cat coding DNA sequences produced identities of 99.5% and 96.4% for *CH1* and *CH2*, respectively (Supplementary Figs. S1 and S2). Pairwise alignments of the DNA sequences with the GenBank Fel d 1 references (M74952.1 and M77341.1) yielded identities ranging from 96.2% to 100% for *CH1* and 93% to 100% for *CH2*. Multisequence alignments of the corresponding protein sequences resulted in identities of 97.9% and 90.1% for chains 1 and 2, respectively (Supplementary Figs. S3 and S4), while pairwise alignments of the protein sequences with the GenBank references (AAC37318.1 and AAC41616.1) yielded identities of 90.3–100% for chain 1 and 84.6–100% for chain 2.

Evolutionary significance of exotic cat Fel d 1 orthologs

Twenty-four exotic (nondomestic) cat genomes representing eight big or wild felid species were examined by BLAST search to identify Fel d 1 orthologous sequences (i.e., homologs separated by speciation; Supplementary Table S2).

The exotic cat species included Chinese mountain cat (*Felis silvestris bieti*; a potential subspecies of the wildcat, *Felis silvestris*^{30–33}), black-footed cat (*Felis nigripes*), leopard cat (*Prionailurus bengalensis*), fishing cat (*Prionailurus viverrinus*), cougar (*Puma concolor*), Iberian lynx (*Lynx pardinus*), Bengal tiger (*Panthera tigris tigris*), and African lion (*Panthera leo*). The subset of exotic cats spans several lineages of the family *Felidae*, ranging from members of the same genus as domestic cat, *Felis*, to *Panthera* species that diverged from domestic cats ~11 million years ago (Fig. 2A).³⁸

Fifty-five unique amino acid substitutions, including 29 substitutions specific to the exotic cat species, were identified from the Fel d 1 protein orthologs (Fig. 2B and Supplementary Table S9). The exotic cat variants were mapped onto the Fel d 1 crystal structure,⁹ which revealed an accumulation of variants at the dimer interface (Fig. 3A). The exotic cat orthologs and corresponding variants are featured in an accompanying interactive visualization (www.feld1viz.com).

Multisequence alignments of the coding DNA orthologs from 24 exotic cats produced identities of 95.9% and 93.5% for *CH1* and *CH2*, respectively (Supplementary Figs. S5 and S6). Pairwise alignments of the DNA orthologs with the GenBank Fel d 1 references yielded identities ranging from 94.6% to 99.3% for *CH1* and from 91.5% to 96.7% for *CH2* (Fig. 2C). Multisequence alignments of the corresponding protein orthologs yielded identities of 92.3% for chain 1 and 86.2% for chain 2 (Supplementary Figs. S7 and S8), while pairwise

alignments of the orthologs with the GenBank references produced identities of 89.1–97.8% and 80.7–90.8% for chains 1 and 2, respectively (Fig. 2C).

A cumulative, multisequence alignment of the DNA orthologs for all domestic and exotic cats analyzed yielded identities of 97.8% for *CH1* and 94.8% for *CH2*. Similarly, a cumulative, multisequence alignment of the protein orthologs produced identities of 94.6% and 87.3% for Fel d 1 chains 1 and 2, respectively. Unrooted, neighbor-joining phylogenies were built from the alignments of the Fel d 1 protein orthologs to evaluate the evolutionary relationships between the domestic and exotic cat species (Fig. 3B). While chain 1 reflected the established evolution of the family *Felidae*, chain 2 deviated from the standard due to greater variability between the orthologs (Figs. 2A and C and 3B).

Editing Fel d 1 with CRISPR-Cas9

The editing efficiencies of each of the 10 CRISPR sgRNAs targeted to either *CH1* (C1G1-6) or *CH2* (C2G1-4) were individually evaluated (Supplementary Table S4). The sgRNAs and Cas9 nuclease were delivered to immortalized feline epithelial cells (CRFK, ATCC CCL-94) as ribonucleoprotein (RNP) complexes using lipid-based transfection. Fel d 1 gene knockout following CRISPR induced frameshift mutations was assessed by DNA sequence decomposition (TIDE³⁶ and ICE) or by enzymatic detection of nucleotide mismatches (T7E1).

Sequence decomposition found CRISPR editing efficiencies of 3.0–54.9% for the 10 Fel d 1-specific sgRNAs (Fig. 4A). Positive control sgRNAs targeted to feline *HPRT1* yielded editing efficiencies of 52.2–77.6% (Supplementary Table S5). The two most efficient Fel d 1 sgRNAs, C1G1 (mean editing efficiency $52.1 \pm 8.3\%$ standard error of the mean [SEM]) and C2G1 (mean editing efficiency $54.9 \pm 10.1\%$ SEM) targeted to *CH1* and *CH2*, respectively, were identified.

The distribution of indels, which range from +2 nucleotide insertions to –17 nucleotide deletions, following editing by CRISPR-Cas9 were evaluated for these two sgRNAs (Fig. 4B). For C1G1, +1 nucleotide insertions or –1 nucleotide deletions accounted for 46% and 21% of all nonzero indels, respectively. By contrast, +1 nucleotide insertions accounted for 89% of the nonzero indels for C2G1. Excluding +1, +0, and –1 nucleotide indels, all other indels were observed at <5% frequency for either sgRNA (Fig. 4B). Indels predicted to produce frameshift mutations in the Fel d 1 protein sequences accounted for 99% and 96% of all nonzero indels for C1G1 and C2G1, respectively.

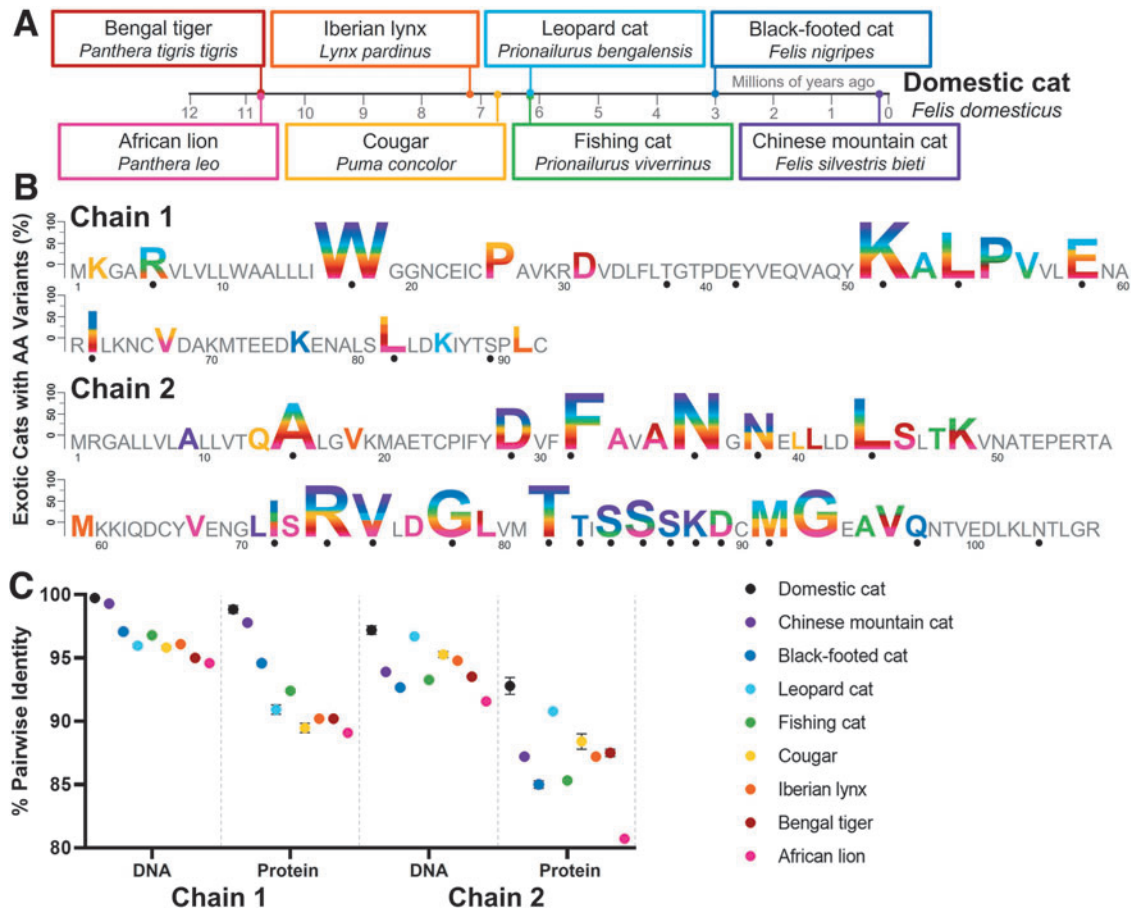


FIG. 2. Comparative sequence analysis of exotic cat Fel d 1 orthologs. Fel d 1 orthologous sequences from 24 exotic cats, representing eight felid species, were analyzed. **(A)** Approximate evolutionary timeline of the domestic and exotic cats.³⁸ **(B)** Amino acid sequences of Fel d 1 chains 1 and 2. The species (color) and percentage (font size) of exotic cats with sequence variants at a given amino acid residue were determined. Corresponding domestic cat variants are indicated (●). **(C)** Mean sequence identities of domestic and exotic cat species following pairwise alignments with the GenBank DNA or protein Fel d 1 references. Data represent the mean $\pm$ standard error of the mean (SEM; 50 domestic cats, 3 cats per exotic species). Color-coded species legend (bottom right) corresponds to panels (A), (B), and (C).

T7E1 analysis found CRISPR editing efficiencies of 6.6–44.9% for the 10 Fel d 1–specific sgRNAs, with mean editing efficiencies of $44.9 \pm 2.5\%$ SEM and $25.5 \pm 1.2\%$ SEM for sgRNAs C1G1 and C2G1, respectively (Fig. 4A and C). The T7E1 assay underestimated the efficiency of C2G1 due to its relatively low variability of indel distribution (Figs. 4A and B). Comparison of the sequence decomposition and T7E1 analyses allowed for the identification and subsequent exclusion of several natural Fel d 1 SNPs from the editing efficiency calculations (Fig. 4C).

Finally, DNA sequence decomposition and T7E1 analysis were used to evaluate off-target CRISPR editing at several predicted potential off-target cleavage sites

(CRISPOR³⁵), which found no evidence of CRISPR editing at the predicted off-target sites due to Fel d 1–specific sgRNAs C1G1 or C2G1 (Fig. 4D).

Discussion and Conclusions

Though the precise biologic function of Fel d 1 remains unknown, our data indicate that the variants or amino acid substitutions are concentrated at the protein dimer interface, suggesting that this region of Fel d 1 is not well conserved and potentially nonessential for the function of the allergen. By contrast, the conserved regions identified as potential target sites for CRISPR-Cas9 editing may prove to be more functionally relevant for Fel d 1.

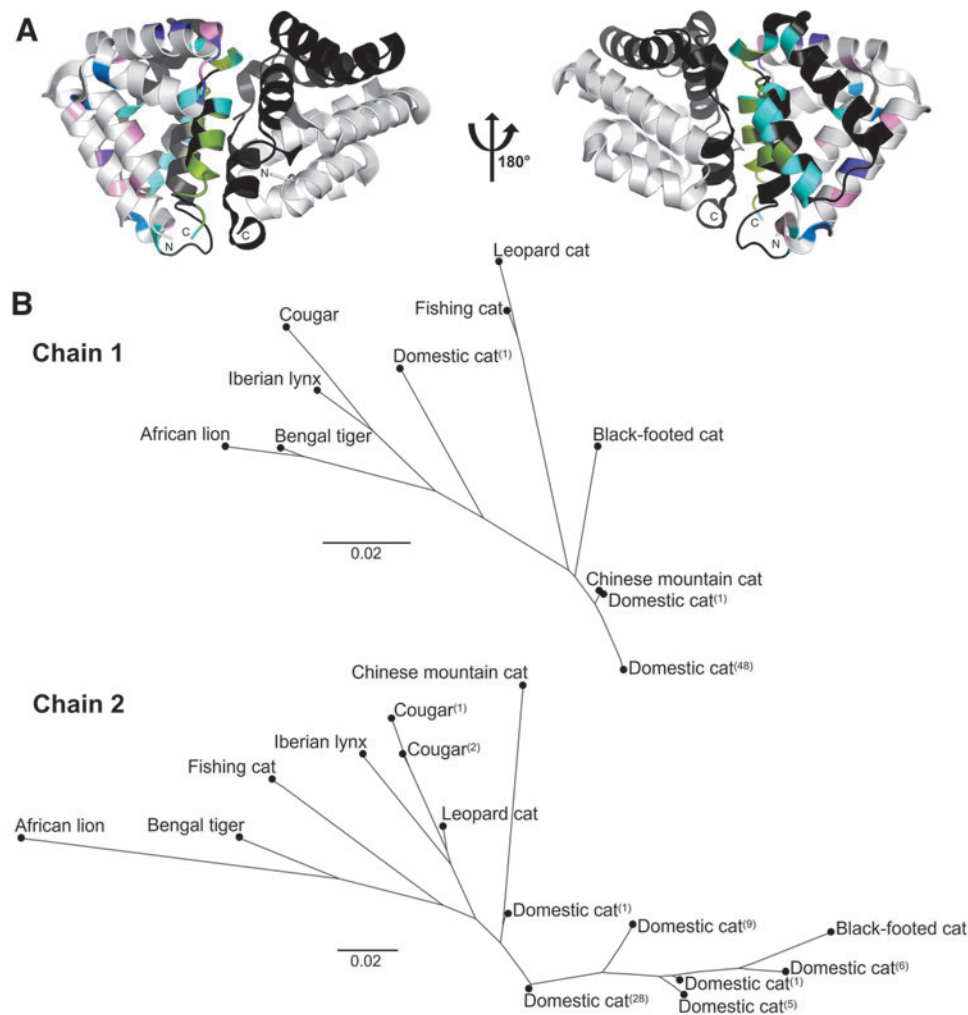


FIG. 3. Evolutionary relationships between domestic and exotic cat Fel d 1 orthologs. **(A)** Ribbon structure of Fel d 1 (PDB 2EJN⁹). Each molecule is a heterodimer of chain 1 (white) and chain 2 (black). The outer molecules show the variants found in domestic cats alone (chain 1: blue; chain 2: yellow), exotic cats alone (chain 1: light pink; chain 2: cyan), or both domestic and exotic cats (chain 1: purple; chain 2: split pea). **(B)** Unrooted phylogenies of Fel d 1 chains 1 (top) and 2 (bottom) assembled from the multisequence alignments of the domestic and exotic cat protein orthologs. Each data point indicates the number of cats for a given species; if no number is specified, the data point represents all cats analyzed for that species (50 domestic cats, 3 cats per exotic species). Scale bars=0.02 aa substitutions per residue.

The variants identified in our study have not been linked to defined levels of Fel d 1 protein expression. However, several of the variable chain 2 residues (Asp29, Phe32, Gly78, Thr82, and Ser85; Supplementary Table S8) were recently recognized as potential binding sites of steroid ligands.³⁹ Our results imply that the ligand-binding function of these residues may be nonessential or that the residues may bind alternative ligands. These interpretations rely on the published structure of recombinant Fel d 1,⁹ as the definitive structure of the natural allergen has yet to be determined.

On average, the chain 2 orthologs were found to be more variable than chain 1 for most of the cat species analyzed, in part due to variability in residues Thr82-Asp89 of chain 2. The *CH2S*⁸ isoform was preferentially encoded by the subset of exotic species closely related to domestic cat, while the more divergent exotic cats favored the canonical *CH2* isoform.

Interestingly, the 50 domestic cat sequences encoded either of the chain 2 isoforms or related variants. A study of RNA derived from domestic cat salivary gland or skin cells proposed that *CH2S* is preferentially

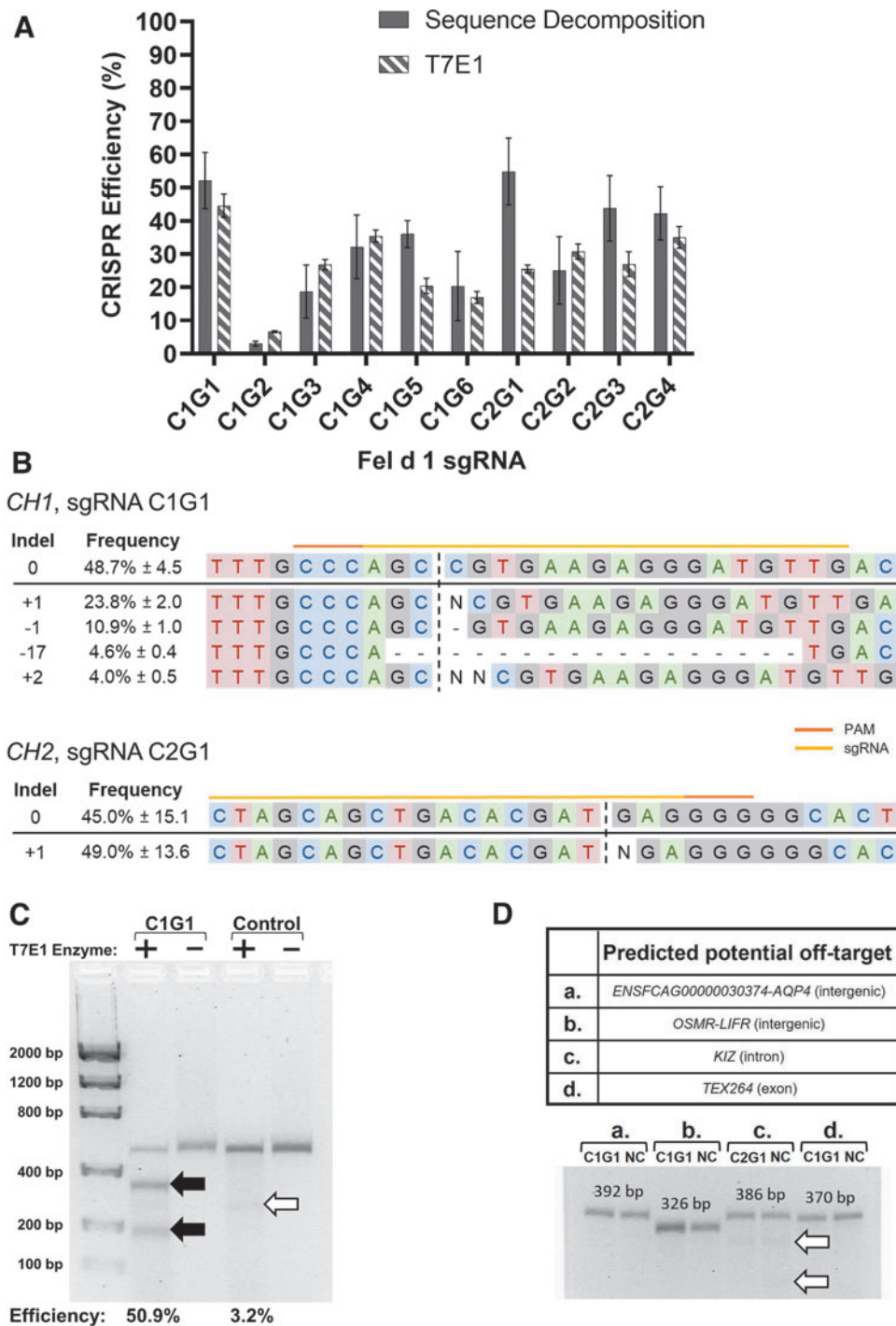


FIG. 4. *In vitro* genome editing of Fel d 1 with CRISPR-Cas9. **(A)** The editing efficiency (%) of 10 Fel d 1-specific CRISPR single-guide RNAs (sgRNAs; *CH1*: C1G1, C1G2, C1G3, C1G4, C1G5, C1G6; *CH2*: C2G1, C2G2, C2G3, C2G4) was evaluated in feline CRFK cells by DNA sequence decomposition and T7 endonuclease 1 (T7E1) analysis. Data represent the mean of three biological replicates ± SEM. **(B)** The distribution of insertions/deletions (indels) was predicted by Inference of CRISPR Edits for C1G1 and C2G1. Indel frequency data represent the mean of three biological replicates ± SEM. Indels with <2% frequency not shown. **(C)** A representative T7E1 agarose gel depicts the parent band (519 bp) and fragment bands (337 and 182 bp, black arrows) for sgRNA C1G1. The negative control shows a T7E1-cleaved SNP (fragments 255 and 264 bp, white arrow), which was excluded from the C1G1 editing efficiency calculation. **(D)** T7E1 analysis found no evidence of off-target CRISPR editing at four predicted potential off-target sites with 3 bp mismatches from the C1G1 or C2G1 target sequences. A T7E1-cleaved SNP (c. *KIZ* gene; white arrows) detected in the experimental and control bands was excluded from the analysis. NC, nontargeting negative control sgRNA.

expressed in the skin, while the canonical *CH2* gene is largely expressed in feline salivary glands.⁸ Tissue-specific expression may partly account for the variability observed in the domestic and exotic cat orthologs, which were sequenced from genomic DNA isolated from blood and tissues that lack Fel d 1 protein expression.

For both chains 1 and 2, the multi- and pairwise sequence alignments of the Fel d 1 orthologs revealed that the DNA sequences exhibit higher identities, or are more similar, than the corresponding protein sequences. More specifically, the data indicate that >70% of the Fel d 1 amino acid variants are the result of nucleotide substitutions in the first or second codon positions, rather than the third position that allows potential redundancy in the subsequent protein sequence.

The ratios of the nonsynonymous and synonymous substitution rates ($\omega = dN/dS$) ranged from 0.5 to 3 for *CH1* and from 0.7 to 4.7 for *CH2* across the eight exotic species analyzed relative to domestic cat (Supplementary Table S3). This suggests that the Fel d 1 genes have been subject to selective pressure in some species, leading to a greater accumulation of nonsynonymous variants.^{34,40–42}

The multisequence alignments suggest that the Fel d 1 gene sequences are not conserved, with the relatively low sequence identities (95.9% and 93.5% for *CH1* and *CH2*, respectively, in 24 exotic cats) roughly mirroring the non-coding, genome-wide averages between species.⁴³ This lack of evolutionary conservation implies that the Fel d 1 genes may be nonessential for cats and suggests that *CH1* and *CH2* are suitable targets for gene deletion.

A protein BLAST search of Fel d 1 chains 1 and 2 in NCBI returns more than 400 significant aligned sequences, ranging in identities from 25% to 100% and representing a broad diversity of mammalian species from the Eutheria, Metatheria, and Prototheria subclasses. One homologous protein specific to the *Mus* genus, androgen-binding protein (ABP), exhibits 35–60% identity with the Fel d 1 sequences. The structural and binding affinity characteristics of Fel d 1 are similar to those of ABP, which is involved in mate selection and chemical communication among mice.^{23,27,39}

Notably, recent studies observed that deleting mouse ABP produced no measurable adverse effects in ABP-deficient mice, indicating that ABP function is likely not essential.⁴⁴ While these studies of a homologous protein suggest that Fel d 1 may function in a similar capacity for cats and may ultimately prove to be nonessential, the definitive test of functionality must be experimental.

Our CRISPR knockouts in immortalized feline cells demonstrate that Fel d 1 is a viable target for gene deletion, which is expected to improve the health of cat allergic patients substantially by effectively removing

the allergen at the source. Several recent approaches to cat allergy aim to neutralize Fel d 1 and to reduce cat allergen exposure.

Cat food supplemented with anti-Fel d 1 polyclonal egg IgY antibody reduced the allergen levels in cat saliva,⁴⁵ resulting in a 47% reduction in Fel d 1 deposited on cats' haircoats from saliva.⁴⁶ Cats immunized with an anti-Fel d 1 vaccine (HypoCatTM) exhibited a 50% reduction in Fel d 1 levels detected in cat tear extracts,⁴⁷ which resulted in a ~30% decrease in symptom severity in nine allergic patients.⁴⁸ Anti-Fel d 1 monoclonal IgG antibodies administered to cat allergic patients competitively blocked IgE binding to the allergen and reduced symptom severity by ~60% in 50% of patients.⁴⁹

Modest reductions in allergen levels may have negligible clinical impact, given that nearly all cat allergic patients experience symptoms at a proposed threshold of 8 μg of Fel d 1 per gram of house dust,⁵⁰ while the allergen may accumulate at levels >1,000 $\mu\text{g/g}$ in house dust.⁵¹

In the future, the CRISPR knockouts will be replicated in Fel d 1-expressing primary feline cells and, eventually, *in vivo* in cats. Future studies will aim to develop a means for deleting the Fel d 1 genes in adult cats and effectively rendering the cats hypoallergenic, while further analyses of homologous proteins from other mammalian species may provide additional insight regarding the precise biologic function of Fel d 1.

Though the current study evaluated the editing efficiencies of individual sgRNAs targeted to either *CH1* or *CH2*, future studies will investigate the efficiency of simultaneous knockouts of the Fel d 1 genes using multiple sgRNAs. Finally, before transitioning to *in vivo* studies, the off-target analysis will be expanded to detect and identify genome-wide double-strand breaks using unbiased screening approaches, such as GUIDE-seq or CIRCLE-seq.^{52,53}

Though CRISPR editing in cats is ostensibly novel, genetic engineering of cats (e.g., selective breeding to create interspecies hybrids⁵⁴) is not unprecedented. Our comparative genomics analysis suggests that Fel d 1 may not be conserved or essential for cats. However, unambiguously deleting Fel d 1 with gene editing technology remains the critical step for identifying the biologic function of the allergen and for determining whether that function is vital for the cats' survival.

To date, this is the most comprehensive bioinformatics analysis of any allergen and the first study to investigate the evolutionary origins of an allergen. Our data illustrate a practical application of CRISPR technology in allergy research or veterinary medicine, and underscore the unique intersection between evolutionary biology, genome engineering, and therapeutic development.

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Author Disclosure Statement

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Supplementary Material

Supplementary Table S1
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Supplementary Table S8
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Supplementary Figure S5
Supplementary Figure S6
Supplementary Figure S7
Supplementary Figure S8

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[illegible]

[illegible]

Domestic_37_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_38_CH1_DNA	AGCKTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_39_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_40_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_41_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_42_CH1_DNA	AGCKTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_43_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_44_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_45_CH1_DNA	AGCKTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_46_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_47_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_48_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_49_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Domestic_50_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
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Fig. S1: Alignment of Fel d 1 CH1 coding DNA sequences from 50 domestic cats. Alignment completed using CLUSTAL Omega. Nucleotides with 100% identity for all 50 cats are marked with an asterisk (*).

[illegible]

[illegible]

Domestic_28_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCWTATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_29_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_30_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYRTATCCARGGYCTTGGATRSACTAGTC	240
Domestic_31_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYRTATCCARGKTCTTGGATGGACTAGTC	240
Domestic_32_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_33_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCWTATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_34_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYKTATCCARGGYCTTGGATRSACTAGTC	240
Domestic_35_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCTTATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_36_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYRTATCCARGGYCTTGGATRSACTAGTC	240
Domestic_37_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYRTATCCARGKYCTTGGATGGACTAGTC	240
Domestic_38_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGATCCAAGKYCTTGGATRSACTAGTC	240
Domestic_39_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYRTATCCARGGYCTTGGATRSACTAGTC	240
Domestic_40_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYKTATCCARGKTCTTGGATGGACTAGTC	240
Domestic_41_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYRTATCCARGGYCTTGGATRSACTAGTC	240
Domestic_42_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGATCCAAGKYCTTGGATRSACTAGTC	240
Domestic_43_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_44_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGATCCAAGTCTTGGATGGACTAGTC	240
Domestic_45_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGATCCAAGTCTTGGATGGACTAGTC	240
Domestic_46_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_47_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTYKTATCCARGGYCTTGGATRSACTAGTC	240
Domestic_48_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_49_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCWTATCCAGGGTCTTGGATGGACTAGTC	240
Domestic_50_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCTTATCCAGGGTCTTGGATGGACTAGTC	240
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Domestic_37_CH2_DNA	ATGATAGCCATCA-----ACGWAKATTGCATGGGTGAAGCAGTTSAGAACACCGTAGAA	294
Domestic_38_CH2_DNA	ATGATAGCCATCA-----ACGTAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Domestic_39_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	300
Domestic_40_CH2_DNA	ATGATAGCCATCA-----ACGTAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Domestic_41_CH2_DNA	ATGATAGCCATCA-----ACGAATATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Domestic_42_CH2_DNA	ATGATAGCCATCA-----ACGTAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Domestic_43_CH2_DNA	ATGACAACCATCAGCTCCAGCAAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	300
Domestic_44_CH2_DNA	ATGATAGCCATCA-----ACGTAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Domestic_45_CH2_DNA	ATGATAGCCATCA-----ACGTAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Domestic_46_CH2_DNA	ATGACAACCATCAGCTCCAGCAAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	300
Domestic_47_CH2_DNA	ATGAYAACCATCAGCTCCAGCAAAGATTGCAYGGGTGAAGCAGTTGAGAACACCGTAGAA	300
Domestic_48_CH2_DNA	ATGACAACCATCAGCTCCAGCAAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	300
Domestic_49_CH2_DNA	ATGACAACCATCAGCTCCAGCAAAGATTGCACGGGTGAAGCAGTTGAGAACACCGTAGAA	300
Domestic_50_CH2_DNA	ATGACAACCATCAGCTCCAGCAAAGATTGCACGGGTGAAGCAGTTGAGAACACCGTAGAA	300

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Domestic_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_2_CH2_DNA	GATCTCAAGCTGAAMACTTTGGGGAGATGA	324
Domestic_3_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_4_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_5_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Domestic_6_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_7_CH2_DNA	GATCTCAAGCTGAAMACTTTGGGGAGATGA	330
Domestic_8_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_9_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_10_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_11_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_12_CH2_DNA	GATCTCAAGCTGAAMACTTTGGGGAGATGA	324
Domestic_13_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_14_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_15_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Domestic_16_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_17_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_18_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_19_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_20_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_21_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_22_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_23_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_24_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_25_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_26_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_27_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_28_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_29_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Domestic_30_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_31_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_32_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_33_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_34_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Domestic_35_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_36_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_37_CH2_DNA	GATCTCAAGCTGAAMACTTTGGGGAGATGA	324
Domestic_38_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_39_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_40_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_41_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Domestic_42_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_43_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_44_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324
Domestic_45_CH2_DNA	GATCTCAAGCTGAAAACCTTTGGGGAGATGA	324

Domestic_46_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_47_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_48_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_49_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Domestic_50_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330

Fig. S2: Alignment of Fel d 1 CH2 coding DNA sequences from 50 domestic cats. Alignment completed using CLUSTAL Omega. Nucleotides with 100% identity for all 50 cats are marked with an asterisk (*).

Table S3: dN/dS ratios of the exotic cat Fel d 1 orthologs. DN=nonsynonymous substitutions, N=nonsynonymous sites, dN=rate of nonsynonymous substitutions, DS=synonymous substitutions, S=synonymous sites, dS=rate of synonymous substitutions. Data indicate one representative cat per exotic species.

CH1	Species	DN	N	dN	DS	S	dS	$\omega=dN/dS$	$\omega=dN/((DS+1)/S)$ Estimate if DS=0
	Chinese mtn cat	2	216.83	0.01	0	59.17	0.00	∞	0.55
	Black-footed cat	6	216.67	0.03	2	59.33	0.03	0.82	-
	Leopard cat	10	217.83	0.05	2	58.17	0.03	1.34	-
	Fishing cat	8	217.83	0.04	1	58.17	0.02	2.14	-
	Cougar	11	216.17	0.05	1	59.83	0.02	3.04	-
	Iberian lynx	11	216.67	0.05	0	59.33	0.00	∞	3.01
	Bengal tiger	9	216.33	0.04	5	59.67	0.08	0.50	-
	African lion	11	216.17	0.05	4	59.83	0.07	0.76	-
CH2	Species	DN	N	dN	DS	S	dS	$\omega=dN/dS$	$\omega=dN/((DS+1)/S)$ Estimate if DS=0
	Chinese mtn cat	14	252.17	0.06	0	68.83	0.00	∞	3.82
	Black-footed cat	17	251.33	0.07	1	69.67	0.01	4.71	-
	Leopard cat	11	251.50	0.04	0	69.50	0.00	∞	3.04
	Fishing cat	16	251.67	0.06	0	69.33	0.00	∞	4.41
	Cougar	14	250.83	0.06	1	70.17	0.01	3.92	-
	Iberian lynx	16	250.67	0.06	1	70.33	0.01	4.49	-
	Bengal tiger	16	251.83	0.06	6	69.17	0.09	0.73	-
	African lion	24	252.33	0.10	3	68.67	0.04	2.18	-

Domestic_10_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_11_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_12_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NXCYMGEAVXNTVEDLKLXTLGR	107
Domestic_13_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_14_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_15_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	107
Domestic_16_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_17_CH2_AA	KIQDCYVENGLXSXXLDGLVMIXI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_18_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_19_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_20_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_21_CH2_AA	KIQDCYVENGLVSKFLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_22_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_23_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_24_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_25_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_26_CH2_AA	KIQDCYVENGLVSKFLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_27_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_28_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_29_CH2_AA	KIQDCYVENGLISXXLDGLVMIAI--NEYCMGEAVQNTVEDLKLNTLGR	107
Domestic_30_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_31_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_32_CH2_AA	KIQDCYVENGLISXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_33_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_34_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	107
Domestic_35_CH2_AA	KIQDCYVENGLISXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_36_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_37_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NXCYMGEAVXNTVEDLKLXTLGR	107
Domestic_38_CH2_AA	KIQDCYVENGLVSKFLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_39_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_40_CH2_AA	KIQDCYVENGLXSXXLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_41_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	107
Domestic_42_CH2_AA	KIQDCYVENGLVSKFLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_43_CH2_AA	KIQDCYVENGLISXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_44_CH2_AA	KIQDCYVENGLVSKFLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_45_CH2_AA	KIQDCYVENGLVSKFLDGLVMIAI--NVDCMGEAVNTVEDLKLKTLGR	107
Domestic_46_CH2_AA	KIQDCYVENGLISXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_47_CH2_AA	KIQDCYVENGLXSXXLDXLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_48_CH2_AA	KIQDCYVENGLISXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_49_CH2_AA	KIQDCYVENGLXSXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109
Domestic_50_CH2_AA	KIQDCYVENGLISXXLDGLVMTTISSSKDCMGEAVQNTVEDLKLNTLGR	109

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Fig. S4: Alignment of Fel d 1 chain 2 protein sequences from 50 domestic cats. Alignment completed using CLUSTAL Omega. Amino acid residues with 100% identity for all 50 cats are marked with an asterisk (*).

cougar_1_CH1_DNA	ATGGAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
cougar_2_CH1_DNA	ATGGAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
cougar_3_CH1_DNA	ATGGAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
bl_foot_1_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
bl_foot_2_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
bl_foot_3_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Ch.mtn_1_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Ch.mtn_2_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Ch.mtn_3_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Ib.lynx_1_CH1_DNA	ATGAAGGGGGCTCATGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Ib.lynx_2_CH1_DNA	ATGAAGGGGGCTCATGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Ib.lynx_3_CH1_DNA	ATGAAGGGGGCTCATGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Af.lion_1_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Af.lion_2_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Af.lion_3_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Be.tiger_1_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Be.tiger_2_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
Be.tiger_3_CH1_DNA	ATGAAGGGGGCTCGTGTTCCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
leop.cat_1_CH1_DNA	ATGAAGGGGGCTTGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
leop.cat_2_CH1_DNA	ATGAAGGGGGCTTGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
leop.cat_3_CH1_DNA	ATGAAGGGGGCTTGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
fishing_1_CH1_DNA	ATGAAGGGGGCTTGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
fishing_2_CH1_DNA	ATGAAGGGGGCTTGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
fishing_3_CH1_DNA	ATGAAGGGGGCTTGTTCTCTGTGCTTCTCTGGGCTGCCTTGCTCTTGATCTCGGGTGGA	60
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Af.lion_3_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
Be.tiger_1_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
Be.tiger_2_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
Be.tiger_3_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
leop.cat_1_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
leop.cat_2_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
leop.cat_3_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
fishing_1_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
fishing_2_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
fishing_3_CH1_DNA	GACGAATATGTTGAGCAAGTGGCACAATACAATGCACGATCTGTGGTGTGGACAATGCC	180
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cougar_1_CH1_DNA	AGAAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
cougar_2_CH1_DNA	AGAAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
cougar_3_CH1_DNA	AGAAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
bl.foot_1_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAACGGAGAATGCTCTC	240
bl.foot_2_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAACGGAGAATGCTCTC	240
bl.foot_3_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAACGGAGAATGCTCTC	240
Ch.mtn_1_CH1_DNA	AGAATACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Ch.mtn_2_CH1_DNA	AGAATACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Ch.mtn_3_CH1_DNA	AGAATACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Ib.lynx_1_CH1_DNA	AGAAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Ib.lynx_2_CH1_DNA	AGAAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Ib.lynx_3_CH1_DNA	AGAAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Af.lion_1_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Af.lion_2_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Af.lion_3_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Be.tiger_1_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Be.tiger_2_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
Be.tiger_3_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
leop.cat_1_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
leop.cat_2_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
leop.cat_3_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
fishing_1_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
fishing_2_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
fishing_3_CH1_DNA	AGAACTGAAGAACTGCATTGATGCAAAAATGACAGAAGAGGATAAGGAGAATGCTCTC	240
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cougar_1_CH1_DNA	AGCGTGCTGGACAAAATATACACAAGTCCTGGGTGTTAA	279
cougar_2_CH1_DNA	AGCGTGCTGGACAAAATATACACAAGTCCTGGGTGTTAA	279
cougar_3_CH1_DNA	AGCGTGCTGGACAAAATATACACAAGTCCTGGGTGTTAA	279
bl.foot_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
bl.foot_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
bl.foot_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Ch.mtn_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Ch.mtn_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Ch.mtn_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Ib.lynx_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Ib.lynx_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Ib.lynx_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Af.lion_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Af.lion_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Af.lion_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Be.tiger_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Be.tiger_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
Be.tiger_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
leop.cat_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
leop.cat_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
leop.cat_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
fishing_1_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
fishing_2_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
fishing_3_CH1_DNA	AGCTTGCTGGACAAAATATACACAAGTCCTCTGTGTTAA	279
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Fig. S5: Alignment of Fel d 1 CH1 coding DNA sequences from 24 exotic cats. Alignment completed using CLUSTAL Omega. Nucleotides with 100% identity for all 24 cats are marked with an asterisk (*).

cougar_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCGTTGCTGGTGACCCGAGAGCTGGGCGTCAAGATG	60
cougar_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCGTTGCTGGTGACCCGAGAGCTGGGCGTCAAGATG	60
cougar_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCGTTGCTGGTGACCCGAGAGCTGGGCGTCAAGATG	60
bl.foot_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGCGCTGGGCGTCAAGATG	60
bl.foot_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGCGCTGGGCGTCAAGATG	60
bl.foot_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGCGCTGGGCGTCAAGATG	60
Ch.mtn_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGACATTGCTGGTGACCCAAGCGCTGGGCGTCAAGATG	60
Ch.mtn_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGACATTGCTGGTGACCCAAGCGCTGGGCGTCAAGATG	60
Ch.mtn_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGACATTGCTGGTGACCCAAGCGCTGGGCGTCAAGATG	60
Ib.lynx_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCTTCAAGATG	60
Ib.lynx_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCTTCAAGATG	60
Ib.lynx_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCTTCAAGATG	60
Af.lion_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
Af.lion_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
Af.lion_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
Be.tiger_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
Be.tiger_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
Be.tiger_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
leop.cat_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
leop.cat_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
leop.cat_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
fishing_1_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
fishing_2_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
fishing_3_CH2_DNA	ATGAGGGGGGCACTGCTTGTGCTGGCATTGCTGGTGACCCAAGAGCTGGGCGTCAAGATG	60
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cougar_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCACGCTTTACTGCGGTGGCCAGTGGAGATGAATTA	120
cougar_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCACGCTTTACTGCGGTGGCCAGTGGAGATGAATTA	120
cougar_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCACGCTTTACTGCGGTGGCCAGTGGAGATGAATTC	120
bl.foot_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATAAAGTCCTTACTGCGGTGGCCAGTGGAGATGAATTA	120
bl.foot_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATAAAGTCCTTACTGCGGTGGCCAGTGGAGATGAATTA	120
bl.foot_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATAAAGTCCTTACTGCGGTGGCCAGTGGAGATGAATTA	120
Ch.mtn_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGAAGTCCTTAATGCGGTGGCCAGTGGAGATGAATTA	120
Ch.mtn_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGAAGTCCTTAATGCGGTGGCCAGTGGAGATGAATTA	120
Ch.mtn_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGAAGTCCTTAATGCGGTGGCCAGTGGAGATGAATTA	120
Ib.lynx_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAGTGAATTA	120
Ib.lynx_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAGTGAATTA	120
Ib.lynx_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAGTGAATTA	120
Af.lion_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCAAGTCCTTACTGTGGTGACCACTGGAAATGAATTA	120
Af.lion_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCAAGTCCTTACTGTGGTGACCACTGGAAATGAATTA	120
Af.lion_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCAAGTCCTTACTGTGGTGACCACTGGAAATGAATTA	120
Be.tiger_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCACGCTTTACTGCGGTGACCTCTGGAAATGAATTA	120
Be.tiger_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCACGCTTTACTGCGGTGACCTCTGGAAATGAATTA	120
Be.tiger_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATCACGCTTTACTGCGGTGACCTCTGGAAATGAATTA	120
leop.cat_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAATGAATTA	120
leop.cat_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAATGAATTA	120
leop.cat_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAATGAATTA	120
fishing_1_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAATGAATTA	120
fishing_2_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAATGAATTA	120
fishing_3_CH2_DNA	GCGGAAACTTGCCCATTTTTTATGACGCTTTACTGCGGTGGCCAGTGGAAATGAATTA	120
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cougar_1_CH2_DNA	CTGTTGGACTCCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
cougar_2_CH2_DNA	CTGTTGGACTCCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
cougar_3_CH2_DNA	CTGTTGGACTCCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
bl.foot_1_CH2_DNA	CTGTTGGACTTCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
bl.foot_2_CH2_DNA	CTGTTGGACTTCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
bl.foot_3_CH2_DNA	CTGTTGGACTTCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Ch.mtn_1_CH2_DNA	CTGTTGGACTTGTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Ch.mtn_2_CH2_DNA	CTGTTGGACTTGTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Ch.mtn_3_CH2_DNA	CTGTTGGACTTGTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180

Ib.lynx_1_CH2_DNA	CTGTTGGACTCCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATCAAA	180
Ib.lynx_2_CH2_DNA	CTGTTGGACTCCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATCAAA	180
Ib.lynx_3_CH2_DNA	CTGTTGGACTCCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATCAAA	180
Af.lion_1_CH2_DNA	CTGTTGGACTTCTTACTCACGGTAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Af.lion_2_CH2_DNA	CTGTTGGACTTCTTACTCACGGTAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Af.lion_3_CH2_DNA	CTGTTGGACTTCTTACTCACGGTAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Be.tiger_1_CH2_DNA	GTGTTGGACTTCTTACTCACGGTAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Be.tiger_2_CH2_DNA	GTGTTGGACTTCTTACTCACGGTAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
Be.tiger_3_CH2_DNA	GTGTTGGACTTCTTACTCACGGTAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
leop.cat_1_CH2_DNA	CTGTTGGACTTCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
leop.cat_2_CH2_DNA	CTGTTGGACTTCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
leop.cat_3_CH2_DNA	CTGTTGGACTTCTCCCTCACAAAAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
fishing_1_CH2_DNA	CTGTTGGACTTCTCCCTCATAAGAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
fishing_2_CH2_DNA	CTGTTGGACTTCTCCCTCATAAGAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180
fishing_3_CH2_DNA	CTGTTGGACTTCTCCCTCATAAGAGTCAATGCTACTGAACCAGAGAGAACAGCCATGAAA	180

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cougar_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAAGGTCTTGGATGCACTAGTC	240
cougar_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAAGGTCTTGGATGCACTAGTC	240
cougar_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAAGGCCTTGGATGCACTAGTC	240
bl.foot_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGTATCCAAGGCCTTGGATACACTAGTC	240
bl.foot_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGTATCCAAGGCCTTGGATACACTAGTC	240
bl.foot_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTTGTATCCAAGGCCTTGGATACACTAGTC	240
Ch.mtn_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGATTTCATATCCAAGTTCTTGGATGGACTAGTC	240
Ch.mtn_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGATTTCATATCCAAGTTCTTGGATGGACTAGTC	240
Ch.mtn_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGATTTCATATCCAAGTTCTTGGATGGACTAGTC	240
Ib.lynx_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCGTATCCACGGGCTTGGATGCACTAGTC	240
Ib.lynx_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCGTATCCACGGGCTTGGATGCACTAGTC	240
Ib.lynx_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCGTATCCACGGGCTTGGATGCACTAGTC	240
Af.lion_1_CH2_DNA	AAAATCCAGGATTGCTACATGGAGAACGGATTGATGGCCAAGGCCTTGGTTGCAATAGTC	240
Af.lion_2_CH2_DNA	AAAATCCAGGATTGCTACATGGAGAACGGATTGATGGCCAAGGCCTTGGWTGCAATAGTC	240
Af.lion_3_CH2_DNA	AAAATCCAGGATTGCTACATGGAGAACGGATTGATGGCCAAGGCCTTGGTTGCAATAGTC	240
Be.tiger_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAAYGGATTGATATCCAAGGTCTTGGATGGAATAGTC	240
Be.tiger_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAAYGGATTGATATCCAAGGTCTTGGATGGAATAGTC	240
Be.tiger_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAAYGGATTGATATCCAAGGTCTTGGATGGAATAGTC	240
leop.cat_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAAGGCCTTGGATGCACTAGTC	240
leop.cat_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAAGGCCTTGGATGCACTAGTC	240
leop.cat_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAAGGCCTTGGATGCACTAGTC	240
fishing_1_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCTTATCCAGGGTCTTGGATGCACTAGTC	240
fishing_2_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCTTATCCAGGGTCTTGGATGCACTAGTC	240
fishing_3_CH2_DNA	AAAATCCAGGATTGCTACGTGGAGAACGGACTCTTATCCAGGGTCTTGGATGCACTAGTC	240

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cougar_1_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACCGTAGAA	300
cougar_2_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACCGTAGAA	300
cougar_3_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACCGTAGAA	300
bl.foot_1_CH2_DNA	ATGATAGCCATC-----AACGAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
bl.foot_2_CH2_DNA	ATGATAGCCATC-----AACGAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
bl.foot_3_CH2_DNA	ATGATAGCCATC-----AACGAAGATTGCATGGGTGAAGCAGTTGAGAACACCGTAGAA	294
Ch.mtn_1_CH2_DNA	ATGATAACCATC-----AACGTAGATTGCATGGTTGAAGCAGTTCAGAACACCGTAGAA	294
Ch.mtn_2_CH2_DNA	ATGATAACCATC-----AACGTAGATTGCATGGTTGAAGCAGTTCAGAACACCGTAGAA	294
Ch.mtn_3_CH2_DNA	ATGATAACCATC-----AACGTAGATTGCATGGTTGAAGCAGTTCAGAACACCGTAGAA	294
Ib.lynx_1_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACTGTAGAA	300
Ib.lynx_2_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACTGTAGAA	300
Ib.lynx_3_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACTGTAGAA	300
Af.lion_1_CH2_DNA	ATGACAACCATCAGCTTCAGCAAAGTTTGCATGGATGAAGCAGTTCAGAACACCGTAGAA	300
Af.lion_2_CH2_DNA	ATGACAACCATCAGCTTCAGCAAAGTTTGCATGGATGAAGCAGTTCAGAACACCGTAGAA	300
Af.lion_3_CH2_DNA	ATGACAACCATCAGCTTCAGCAAAGTTTGCATGGATGAAGCAGTTCAGAACACCGTAGAA	300
Be.tiger_1_CH2_DNA	ATGGCAACCATCAGCTCCAGCAAAGACTGCATGGGTGAAGCAGTTCAGAACACCGTAGAA	300
Be.tiger_2_CH2_DNA	ATGGCAACCATCAGCTCCAGCAAAGACTGCATGGGTGAAGCAGTTCAGAACACCGTAGAA	300
Be.tiger_3_CH2_DNA	ATGGCAACCATCAGCTCCAGCAAAGACTGCATGGGTGAAGCAGTTCAGAACACCGTAGAA	300

leop.cat_1_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACCGTAGAA	300
leop.cat_2_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACCGTAGAA	300
leop.cat_3_CH2_DNA	ATGATAACCATCAGCTCCAGCAAAGATTGCATCGTTGAAGCAGTTCAGAACACCGTAGAA	300
fishing_1_CH2_DNA	ATGGCAACCATC-----AGCAAAGTTTGATCGTTGAAACAAGTCAGAACACCGTAGAA	294
fishing_2_CH2_DNA	ATGGCAACCATC-----AGCAAAGTTTGAKCGTTGAAACAAGTCAGAACACCGTAGAA	294
fishing_3_CH2_DNA	ATGGCAACCATC-----AGCAAAGTTTGATCGTTGAAACAAGTCAGAACACCGTAGAA	294
	*** * ***** * * ** **** * **** * * ***** *	
cougar_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
cougar_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
cougar_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
b1.foot_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
b1.foot_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
b1.foot_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Ch.mtn_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Ch.mtn_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Ch.mtn_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
Ib.lynx_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Ib.lynx_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Ib.lynx_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Af.lion_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Af.lion_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Af.lion_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Be.tiger_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Be.tiger_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
Be.tiger_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
leop.cat_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
leop.cat_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
leop.cat_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	330
fishing_1_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
fishing_2_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324
fishing_3_CH2_DNA	GATCTCAAGCTGAACACTTTGGGGAGATGA	324

cougar_1_CH1_AA	MEGARVLVLLWAALLLISGGNCEICSAVKRDVDFLTGTDPDEYVEQVAQYNARPVVLANA	60
cougar_2_CH1_AA	MEGARVLVLLWAALLLISGGNCEICSAVKRDVDFLTGTDPDEYVEQVAQYNARPVVLANA	60
cougar_3_CH1_AA	MEGARVLVLLWAALLLISGGNCEICSAVKRDVDFLTGTDPDEYVEQVAQYNARPVVLANA	60
bl.foot_1_CH1_AA	MKGARVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYSALSVVLENA	60
bl.foot_2_CH1_AA	MKGARVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYSALSVVLENA	60
bl.foot_3_CH1_AA	MKGARVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYSALSVVLENA	60
Ch.mtn_1_CH1_AA	MKGARVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNALPVVLENA	60
Ch.mtn_2_CH1_AA	MKGARVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNALPVVLENA	60
Ch.mtn_3_CH1_AA	MKGARVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNALPVVLENA	60
Ib.lynx_1_CH1_AA	MKGAVHLVLLWAALLLISGGNCEICSAVKRDVDFLTGTDPDEYVEQVAQYNASPVVLANA	60
Ib.lynx_2_CH1_AA	MKGAVHLVLLWAALLLISGGNCEICSAVKRDVDFLTGTDPDEYVEQVAQYNASPVVLANA	60
Ib.lynx_3_CH1_AA	MKGAVHLVLLWAALLLISGGNCEICSAVKRDVDFLTGTDPDEYVEQVAQYNASPVVLANA	60
Af.lion_1_CH1_AA	MKGARVLVLLWAALLLISGGNCEICSAVKRIVDFLTGTDPDEYVEQVAQYNARSVVLDNA	60
Af.lion_2_CH1_AA	MKGARVLVLLWAALLLISGGNCEICSAVKRIVDFLTGTDPDEYVEQVAQYNARSVVLDNA	60
Af.lion_3_CH1_AA	MKGARVLVLLWAALLLISGGNCEICSAVKRIVDFLTGTDPDEYVEQVAQYNARSVVLDNA	60
Be.tiger_1_CH1_AA	MKGARVLVLLWAALLLISGGNCEICSAVKRNVDFLTGTDPDEYVEQVAQYNARSVVLDNA	60
Be.tiger_2_CH1_AA	MKGARVLVLLWAALLLISGGNCEICSAVKRNVDFLTGTDPDEYVEQVAQYNARSVVLDNA	60
Be.tiger_3_CH1_AA	MKGARVLVLLWAALLLISGGNCEICSAVKRNVDFLTGTDPDEYVEQVAQYNARSVVLDNA	60
leop.cat_1_CH1_AA	MKGACVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNTESEVLKNA	60
leop.cat_2_CH1_AA	MKGACVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNTESEVLKNA	60
leop.cat_3_CH1_AA	MKGACVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNTESEVLKNA	60
fishing_1_CH1_AA	MKGACVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNTESEVLENA	60
fishing_2_CH1_AA	MKGACVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNTESEVLENA	60
fishing_3_CH1_AA	MKGACVLVLLWAALLLISGGNCEICPAVKRDVDFLTGTDPDEYVEQVAQYNTESEVLENA	60
	. * ***** ** *	
cougar_1_CH1_AA	RKLKNCIDAKMTEEDKENALSVLDKIYTSPGC	92
cougar_2_CH1_AA	RKLKNCIDAKMTEEDKENALSVLDKIYTSPGC	92
cougar_3_CH1_AA	RKLKNCVDAKMTEEDKENALSVLDKIYTSPGC	92
bl.foot_1_CH1_AA	RNLKNCVDAKMTEEDTENALSLLDKIYTSPLC	92
bl.foot_2_CH1_AA	RNLKNCVDAKMTEEDTENALSLLDKIYTSPLC	92
bl.foot_3_CH1_AA	RNLKNCVDAKMTEEDTENALSLLDKIYTSPLC	92
Ch.mtn_1_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
Ch.mtn_2_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
Ch.mtn_3_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
Ib.lynx_1_CH1_AA	RKLKNCVDAKMTEEDKENALSVLDKIYTSPGC	92
Ib.lynx_2_CH1_AA	RKLKNCVDAKMTEEDKENALSVLDKIYTSPGC	92
Ib.lynx_3_CH1_AA	RKLKNCVDAKMTEEDKENALSVLDKIYTSPGC	92
Af.lion_1_CH1_AA	RTLKNCADAKMTEEDKENALSVLDKIYTSPLC	92
Af.lion_2_CH1_AA	RTLKNCADAKMTEEDKENALSVLDKIYTSPLC	92
Af.lion_3_CH1_AA	RTLKNCADAKMTEEDKENALSVLDKIYTSPLC	92
Be.tiger_1_CH1_AA	RTLKNCVDAKMTEEDKENALSVLDKIYTSPLC	92
Be.tiger_2_CH1_AA	RTLKNCVDAKMTEEDKENALSVLDKIYTSPLC	92
Be.tiger_3_CH1_AA	RTLKNCVDAKMTEEDKENALSVLDKIYTSPLC	92
leop.cat_1_CH1_AA	RILKNCVDAKMTEEDKENALSLLDRIYTSPLC	92
leop.cat_2_CH1_AA	RILKNCVDAKMTEEDKENALSLLDRIYTSPLC	92
leop.cat_3_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
fishing_1_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
fishing_2_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
fishing_3_CH1_AA	RILKNCVDAKMTEEDKENALSLLDKIYTSPLC	92
	* **** ***** ** *	

cougar_1_CH2_AA	MRGALLVLALLVTRELGVKMAETCPIFYHVF	TAVASGDELLLDSSLTKVNATEPERTAMK	60
cougar_2_CH2_AA	MRGALLVLALLVTRELGVKMAETCPIFYHVF	TAVASGDELLLDSSLTKVNATEPERTAMK	60
cougar_3_CH2_AA	MRGALLVLALLVTRELGVKMAETCPIFYHVF	TAVASGDEFLLDSSLTKVNATEPERTAMK	60
bl.foot_1_CH2_AA	MRGALLVLALLVTQALGVKMAETCPIFYKVF	TAVASGDELLLDLDFSLTKVNATEPERTAMK	60
bl.foot_2_CH2_AA	MRGALLVLALLVTQALGVKMAETCPIFYKVF	TAVASGDELLLDLDFSLTKVNATEPERTAMK	60
bl.foot_3_CH2_AA	MRGALLVLALLVTQALGVKMAETCPIFYKVF	TAVASGDELLLDLDFSLTKVNATEPERTAMK	60
Ch.mtn_1_CH2_AA	MRGALLVLTLLVTQALGVKMAETCPIFYEVF	NAVASGDELLLDLDSLTKVNATEPERTAMK	60
Ch.mtn_2_CH2_AA	MRGALLVLTLLVTQALGVKMAETCPIFYEVF	NAVASGDELLLDLDSLTKVNATEPERTAMK	60
Ch.mtn_3_CH2_AA	MRGALLVLTLLVTQALGVKMAETCPIFYEVF	NAVASGDELLLDLDSLTKVNATEPERTAMK	60
Ib.lynx_1_CH2_AA	MRGALLVLALLVTQELGFKMAETCPIFYDVF	TAVASGSELLLDSSLTKVNATEPERTAIK	60
Ib.lynx_2_CH2_AA	MRGALLVLALLVTQELGFKMAETCPIFYDVF	TAVASGSELLLDSSLTKVNATEPERTAIK	60
Ib.lynx_3_CH2_AA	MRGALLVLALLVTQELGFKMAETCPIFYDVF	TAVASGSELLLDSSLTKVNATEPERTAIK	60
Af.lion_1_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYQVF	TVVTTGNELLDLDFLLTVVNATEPERTAMK	60
Af.lion_2_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYQVF	TVVTTGNELLDLDFLLTVVNATEPERTAMK	60
Af.lion_3_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYQVF	TVVTTGNELLDLDFLLTVVNATEPERTAMK	60
Be.tiger_1_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYHVF	TAVTSGNELVLDLDFLLTVVNATEPERTAMK	60
Be.tiger_2_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYHVF	TAVTSGNELVLDLDFLLTVVNATEPERTAMK	60
Be.tiger_3_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYHVF	TAVTSGNELVLDLDFLLTVVNATEPERTAMK	60
leop.cat_1_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYDVF	TAVASGNELLDLDFSLTKVNATEPERTAMK	60
leop.cat_2_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYDVF	TAVASGNELLDLDFSLTKVNATEPERTAMK	60
leop.cat_3_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYDVF	TAVASGNELLDLDFSLTKVNATEPERTAMK	60
fishing_1_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYDVF	TAVASGNELLDLDFSLIRVNATEPERTAMK	60
fishing_2_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYDVF	TAVASGNELLDLDFSLIRVNATEPERTAMK	60
fishing_3_CH2_AA	MRGALLVLALLVTQELGVKMAETCPIFYDVF	TAVASGNELLDLDFSLIRVNATEPERTAMK	60
	*****:****: ** *****:*.:.*:.*:.* * *****:*		
cougar_1_CH2_AA	KIQDCYVENGLISKVLDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
cougar_2_CH2_AA	KIQDCYVENGLISKVLDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
cougar_3_CH2_AA	KIQDCYVENGLISKALDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
bl.foot_1_CH2_AA	KIQDCYVENGLVSKALDTLVMIAIN--EDCMGEAVENTVEDLKLNTLGR	107	
bl.foot_2_CH2_AA	KIQDCYVENGLVSKALDTLVMIAIN--EDCMGEAVENTVEDLKLNTLGR	107	
bl.foot_3_CH2_AA	KIQDCYVXNGLVSKALDTLVMIAIN--EDCMGEAVENTVEDLKLNTLGR	107	
Ch.mtn_1_CH2_AA	KIQDCYVENGFISKFLDGLVMITIN--VDCMVEAVQNTVEDLKLNTLGR	107	
Ch.mtn_2_CH2_AA	KIQDCYVENGFISKFLDGLVMITIN--VDCMVEAVQNTVEDLKLNTLGR	107	
Ch.mtn_3_CH2_AA	KIQDCYVENGFISKFLDGLVMITIN--VDCMVEAVQNTVEDLKLNTLGR	107	
Ib.lynx_1_CH2_AA	KIQDCYVENGLVSTGLDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
Ib.lynx_2_CH2_AA	KIQDCYVENGLVSTGLDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
Ib.lynx_3_CH2_AA	KIQDCYVENGLVSTGLDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
Af.lion_1_CH2_AA	KIQDCYMENGLMAKALVAIVMTTISFSKVCMDAAQNTVEDLKLNTLGR	109	
Af.lion_2_CH2_AA	KIQDCYMENGLMAKALVAIVMTTISFSKVCMDAAQNTVEDLKLNTLGR	109	
Af.lion_3_CH2_AA	KIQDCYMENGLMAKALVAIVMTTISFSKVCMDAAQNTVEDLKLNTLGR	109	
Be.tiger_1_CH2_AA	KIQDCYVEXGLISKVLDGIVMATISSSKDCMGEAAQNTVEDLKLNTLGR	109	
Be.tiger_2_CH2_AA	KIQDCYVENGLISKVLDGIVMATISSSKDCMGEAAQNTVEDLKLNTLGR	109	
Be.tiger_3_CH2_AA	KIQDCYVEXGLISKVLDGIVMATISSSKDCMGEAAQNTVEDLKLNTLGR	109	
leop.cat_1_CH2_AA	KIQDCYVENGLISKALDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
leop.cat_2_CH2_AA	KIQDCYVENGLISKALDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
leop.cat_3_CH2_AA	KIQDCYVENGLISKALDALVMITISSSKDCIVEAVQNTVEDLKLNTLGR	109	
fishing_1_CH2_AA	KIQDCYVENGLLSRVLDALVMATIS--KVCIVETSQNTVEDLKLNTLGR	107	
fishing_2_CH2_AA	KIQDCYVENGLLSRVLDALVMATIS--KVCIVETSQNTVEDLKLNTLGR	107	
fishing_3_CH2_AA	KIQDCYVENGLLSRVLDALVMATIS--KVCIVETSQNTVEDLKLNTLGR	107	
	*****: *::: * :*: :*. * *: :*****		

Fig. S8: Alignment of Fel d 1 chain 2 protein sequences from 24 exotic cats. Alignment completed using CLUSTAL Omega. Amino acid residues with 100% identity for all 24 cats are marked with an asterisk (*).

Table S1: PCR amplification of domestic cat Fel d 1 *CH1* and *CH2*.

DNA Target Amplification	Target	Primer	Primer Sequence	
	<i>CH1</i> (full length)	forward	CCTGCCCACACATACATCTCC	
		reverse	GTAAGACACCTGGACATGGCA	
	<i>CH2</i> (full length)	forward	CCTCTCCAAGAGCTTTGTCCTC	
		reverse	GTCAGCCTGGATTGAGAGTG	
PCR Conditions	Denature	Anneal	Extend	Repeat
	95°C for 30s	64°C for 30s	72°C for 2 min	29 cycles

Table S2: Exotic cat genomes analyzed for Fel d 1 orthologous sequences.

Species	Common Name	BioSample	SRA Experiments
<i>Puma concolor</i>	Cougar	SAMN09396574	SRX4503205, SRX4503206
		SAMN09396599	SRX4521342, SRX4521343
		SAMN09396547	SRX4475768, SRX4475769
<i>Felis nigripes</i>	Black-footed cat	SAMN04100112	SRX1284341, SRX1284342
		SAMN04100110	SRX1284343, SRX1284344
		SAMN07678084	SRX8008500
<i>Felis silvestris bieti</i>	Chinese mountain cat	SAMN09509265	SRX4485362
		SAMN09509266	SRX4485361
		SAMN09509267	SRX4485353
<i>Lynx pardinus</i>	Iberian lynx	SAMEA3862499	ERX1327176, ERX1327177 ERX1327178, ERX1327179
		SAMEA3862504	ERX1327196, ERX1327197 ERX1327198, ERX1327199
		SAMEA3862505	ERX1327200, ERX1327201
			ERX1327202, ERX1327203
<i>Panthera leo</i>	African lion	SAMN02086970	SRX273035
		SAMN12375718	SRX6809195, SRX6809196 SRX6748111, SRX6787721
			ERX4107161, ERX4107167
		SAMEA5374043	ERX4107173, ERX4107179 ERX4107185, ERX4107191
<i>Panthera tigris tigris</i>	Bengal tiger	SAMN09080448	SRX4071869
		SAMN09080471	SRX4071849
		SAMN07160631	SRX2848113, SRX2848114 SRX2848115
<i>Prionailurus bengalensis</i>	Leopard cat	SAMN03771693	SRX1058464
		SAMN05913184	SRX2248195
		SAMN05913190	SRX2248207
<i>Prionailurus viverrinus</i>	Fishing cat	SAMN07666179	SRX3213489
		SAMN05913178	SRX2248197
		SAMN15669430	SRX8880248, SRX8880249 SRX8880250, SRX8880251

Table S3: dN/dS ratios of the exotic cat Fel d 1 orthologs. DN=nonsynonymous substitutions, N=nonsynonymous sites, dN=rate of nonsynonymous substitutions, DS=synonymous substitutions, S=synonymous sites, dS=rate of synonymous substitutions. Data indicate one representative cat per exotic species.

CH1	Species	DN	N	dN	DS	S	dS	$\omega=dN/dS$	$\omega=dN/((DS+1)/S)$ Estimate if DS=0
	Chinese mtn cat	2	216.83	0.01	0	59.17	0.00	∞	0.55
	Black-footed cat	6	216.67	0.03	2	59.33	0.03	0.82	-
	Leopard cat	10	217.83	0.05	2	58.17	0.03	1.34	-
	Fishing cat	8	217.83	0.04	1	58.17	0.02	2.14	-
	Cougar	11	216.17	0.05	1	59.83	0.02	3.04	-
	Iberian lynx	11	216.67	0.05	0	59.33	0.00	∞	3.01
	Bengal tiger	9	216.33	0.04	5	59.67	0.08	0.50	-
	African lion	11	216.17	0.05	4	59.83	0.07	0.76	-
CH2	Species	DN	N	dN	DS	S	dS	$\omega=dN/dS$	$\omega=dN/((DS+1)/S)$ Estimate if DS=0
	Chinese mtn cat	14	252.17	0.06	0	68.83	0.00	∞	3.82
	Black-footed cat	17	251.33	0.07	1	69.67	0.01	4.71	-
	Leopard cat	11	251.50	0.04	0	69.50	0.00	∞	3.04
	Fishing cat	16	251.67	0.06	0	69.33	0.00	∞	4.41
	Cougar	14	250.83	0.06	1	70.17	0.01	3.92	-
	Iberian lynx	16	250.67	0.06	1	70.33	0.01	4.49	-
	Bengal tiger	16	251.83	0.06	6	69.17	0.09	0.73	-
	African lion	24	252.33	0.10	3	68.67	0.04	2.18	-

Table S4: CRISPR sgRNAs targeted to Fel d 1 genes *CH1* or *CH2*.

sgRNA	Genomic Target	Target DNA Sequence	sgRNA Sequence
C1G1	Fel d 1 <i>CH1</i>	CAACATCCCTCTTCACGGCT	CAACAUCCCUCUUCACGGCU
C1G2	Fel d 1 <i>CH1</i>	TCAACATCCCTCTTCACGGC	UCAACAUCCCUCUUCACGGC
C1G3	Fel d 1 <i>CH1</i>	AAATTTGCCAGCCGTGAAG	AAAUUUGCCCAGCCGUGAAG
C1G4	Fel d 1 <i>CH1</i>	GCTGGGCAAATTTACAATC	GCUGGGCAAUUUCACAAUC
C1G5	Fel d 1 <i>CH1</i>	AATTTGCCAGCCGTGAAGA	AAUUUGCCCAGCCGUGAAGA
C1G6	Fel d 1 <i>CH1</i>	CGACGAATATGTTGAGCAAG	CGACGAAUAUGUUGAGCAAG
C2G1	Fel d 1 <i>CH2</i>	CTAGCAGCTGACACGATGAG	CUAGCAGCUGACACGAUGAG
C2G2	Fel d 1 <i>CH2</i>	TAGCAGCTGACACGATGAGG	UAGCAGCUGACACGAUGAGG
C2G3	Fel d 1 <i>CH2</i>	ACTGCTTGTGCTGGCATTGC	ACUGCUUGUGCUGGCAUUGC
C2G4	Fel d 1 <i>CH2</i>	AGCAGCTGACACGATGAGGG	AGCAGCUGACACGAUGAGGG

Table S5: CRISPR positive control sgRNAs targeted to feline *HPRT1*.

sgRNA	Target DNA Sequence	sgRNA Sequence	CRISPR Efficiency (%) Mean of two replicates
HPRT1-1	GCTTGCACGAGATGTGATGA	GCUUGCACGAGAUGUGAUGA	52.20%
HPRT1-3	CAAGCAGTATAATCCAAAGA	CAAGCAGUAUAAUCCAAAGA	67.47%
HPRT1-4	TAATTATGGACAGGACGGAA	UAAUUAUGGACAGGACGGAA	61.23%
HPRT1-5	CTGTAATGACCAGTCAACAG	CUGUAAUGACCAGUCAACAG	77.57%

Table S6: PCR amplification of *CH1* and *CH2* CRISPR cut sites.

DNA Target Amplification	Target	Primer	Primer Sequence	
	CH1 (CRISPR cut site)	forward	GGTGGAAGTAGGTGTCTGGG	
		reverse	TCTTCCCCACATGATCCCCTC	
	CH2 (CRISPR cut site)	forward	CCTCTCCAAGAGCTTTGTCCTC	
		reverse	GCACCACTACCCCCAACTAA	
PCR Conditions	Denature	Anneal	Extend	Repeat
	95°C for 30s	63°C for 30s	72°C for 1 min	40 cycles

Table S7: PCR amplification of predicted potential off-targets for sgRNAs C1G1 and C2G1.

DNA Amplification	Potential Off-Target	Corresponding Fel d 1 sgRNA	Primer	Primer Sequence	
	<i>ENSFCAG030374 - AQP4</i>	C1G1	forward	GCCTGCATACAACCTGGTCT	
			reverse	GAGCAATGCCAACGACAGTG	
	<i>OSMR - LIFR</i>	C1G1	forward	AGCTGCATCTGTTTGGGACA	
			reverse	TATATGGCGGCGGTTGTCAG	
	<i>KIZ</i>	C2G1	forward	GGTTGTGTCACTCGGGTTCT	
			reverse	GCCTTGCAGAAGCCACTAGA	
	<i>TEX264</i>	C2G1	forward	TAAGGCCTCTGTCCTCTCCC	
			reverse	TACTCCTCACCTTCTCCGG	
PCR Conditions	Denature	Anneal		Extend	Repeat
	95°C for 30s	60-64°C for 30s		72°C for 1 min	40 cycles

Table S8: Fel d 1 protein variants identified from 50 domestic cats.

Patterns of correlated variants are indicated by color. All variants are the result of SNPs unless noted: rare mutation⁽¹⁾, or MNP⁽²⁾. Novel variants are underlined.

	Residue	Variant	Frequency	Structure Location ¹
Chain 1	Arg5	R → C	20%	Signal peptide
	Trp18	W → S	24%	Signal peptide
	Thr37	T → <u>M</u> ⁽¹⁾	2%	H1 alpha helix
	Glu42	E → <u>K</u> ⁽¹⁾	2%	H2 alpha helix
	Lys51	K → N	28%	H2 - H3 interface
	Leu53	L → <u>R</u> ⁽¹⁾	2%	H2 - H3 interface
	Glu58	E → <u>A</u> ⁽¹⁾	2%	H3 alpha helix
	Ile62	I → <u>N</u> ⁽¹⁾	2%	H3 alpha helix
	Leu82	L → V	26%	H4 alpha helix
	Ser89	S → <u>N</u> ⁽¹⁾	2%	H4 - H5 interface
Chain 2	Ala15	A → <u>E</u>	44%	Signal peptide
	Asp29	D → <u>E</u>	12%	H5 alpha helix
	Phe32	F → T; <u>T or S</u> ⁽¹⁾ ; <u>F or T or S or I</u> ⁽²⁾	16%; 2%; 16%	H5 alpha helix
	Asn36	N → <u>S</u>	34%	Ca++ ion binding
	Asn38	N → <u>D</u>	14%	H5 - H6 interface
	Leu44	L → <u>L or F or S</u> ⁽²⁾	20%	H6 alpha helix
	Ile72	I → L; V; L or V	20%; 40%; 26%	Ca++ ion binding
	Arg74	R → K	66%	H7 - H8 interface
	Val75	V → F; <u>A; V or F or A or S</u> ⁽²⁾	30%; 28%; 8%	H7 - H8 interface
	Gly78	G → <u>A; G or A or T or R</u> ⁽²⁾	4%; 32%	H8 alpha helix
	Thr82	T → I	64%	H8 alpha helix
	Thr83	T → A	44%	H8 alpha helix
	Ser85	S → -	42%	H8 alpha helix
	Ser86	S → -	42%	Post H8 alpha helix
	Ser87	S → N	42%	Post H8 alpha helix
	Lys88	K → E; <u>V; E or V</u>	10%; 26%; 6%	Post H8 alpha helix
	Asp89	D → Y	16%	Post H8 alpha helix
	Met91	M → T	30%	Post H8 alpha helix
	Gln96	Q → E	34%	Post H8 alpha helix
	Asn105	N → K	34%	Post H8 alpha helix

Reference:

1. Kaiser L, Velickovic TC, Badia-Martinez D et al. Structural characterization of the tetrameric form of the major cat allergen Fel d 1. *J Mol Biol.* 2007;370:714-727. DOI: 10.1016/j.jmb.2007.04.074

Table S9: Protein sequence variants identified from Fel d 1 orthologs of 24 exotic cats.

Species: Chinese mountain cat (Ch.mtn), black-footed cat (bl.foot), leopard cat (leop.cat), fishing cat (fishing), cougar, Iberian lynx (lb.lynx), Bengal tiger (Be.tiger), African lion (Af.lion)

	Residue	Variant	Frequency (% of all exotic cats analyzed)	Exotic Cat Species
Chain 1	Lys2	K→E	12.5%	cougar
	Arg5	R→C; R→H	25%; 12.5%	leop.cat, fishing; lb.lynx
	Trp18	W→S	100%	cougar, bl.foot, Ch.mtn, lb.lynx, Af.lion, Be.tiger, leop.cat, fishing
	Pro26	P→S	50%	cougar, lb.lynx, Af.lion, Be.tiger
	Asp31	D→I; D→N	12.5%; 12.5%	Af.lion; Be.tiger
	Lys51	K→N; K→S	87.5%; 12.5%	cougar, Ch.mtn, lb.lynx, Af.lion, Be.tiger, leop.cat, fishing; bl.foot
	Ala52	A→T	25%	leop.cat, fishing
	Leu53	L→S; L→E; L→R	12.5%; 25%; 37.5%	lb.lynx; leop.cat, fishing; cougar, Af.lion, Be.tiger
	Pro54	P→S	62.5%	bl.foot, Af.lion, Be.tiger, leop.cat, fishing
	Val55	V→E	25%	leop.cat, fishing
	Glu58	E→A; E→D; E→K	25%; 25%; 8.3%	cougar, lb.lynx; Af.lion, Be.tiger; leop.cat
	Ile62	I→N; I→K; I→T	12.5%; 25%; 25%	bl.foot; cougar, lb.lynx; Af.lion, Be.tiger
	Val67	V→I; V→A	8.3%; 12.5%	cougar; Af.lion
	Lys76	K→T	12.5%	bl.foot
	Leu82	L→V	50%	cougar, lb.lynx, Af.lion, Be.tiger
Chain 2	Lys85	K→R	8.3%	leop.cat
	Leu91	L→G	25%	cougar, lb.lynx
	Ala9	A→T	12.5%	Ch.mtn
	Gln14	Q→R	12.5%	cougar
	Ala15	A→E	75%	cougar, lb.lynx, Af.lion, Be.tiger, leop.cat, fishing
	Val18	V→F	12.5%	lb.lynx
	Asp29	D→E; D→H; D→K; D→Q	12.5%; 25%; 12.5%; 12.5%	Ch.mtn; cougar, Be.tiger; bl.foot; Af.lion
	Phe32	F→T; F→N	87.5%; 12.5%	cougar, bl.foot, lb.lynx, Af.lion, Be.tiger, leop.cat, fishing; Ch.mtn
	Ala33	A→V	12.5%	Af.lion
	Ala35	A→T	25%	Af.lion, Be.tiger
	Asn36	N→S; N→T	87.5%; 12.5%	cougar, bl.foot, Ch.mtn, lb.lynx, Be.tiger, leop.cat, fishing; Af.lion
	Asn38	N→D; N→S	37.5%; 12.5%	cougar, bl.foot, Ch.mtn; lb.lynx
	Leu40	L→F	4.2% (one individual)	cougar
	Leu41	L→V	12.5%	Be.tiger
	Leu44	L→F; L→S	62.5%; 25%	bl.foot, Af.lion, Be.tiger, leop.cat, fishing; cougar, lb.lynx
	Ser45	S→L	25%	Af.lion, Be.tiger
	Thr47	T→I	12.5%	fishing
	Lys48	K→V; K→R	25%; 12.5%	Af.lion, Be.tiger; fishing
	Met59	M→I	12.5%	lb.lynx
	Val67	V→M	12.5%	Af.lion
	Leu71	L→F	12.5%	Ch.mtn
	Ile72	I→L; I→V; I→M	12.5%; 25%; 12.5%	fishing; bl.foot, lb.lynx; Af.lion
	Ser73	S→A	12.5%	Af.lion
	Arg74	R→K; R→T	75%; 12.5%	cougar, bl.foot, Ch.mtn, Af.lion, Be.tiger, leop.cat; lb.lynx
	Val75	V→A; V→F; V→G	41.7%; 12.5%; 12.5%	cougar, bl.foot, Af.lion, leop.cat; Ch.mtn; lb.lynx
	Asp77	D→V	12.5%	Af.lion
	Gly78	G→A; G→T	62.5%; 12.5%	cougar, lb.lynx, Af.lion, leop.cat, fishing; bl.foot
	Leu79	L→I	25%	Af.lion, Be.tiger
	Thr82	T→I; T→A	62.5%; 25%	cougar, bl.foot, Ch.mtn, lb.lynx, leop.cat; Be.tiger, fishing
	Thr83	T→A	12.5%	bl.foot
	Ser85	S→-	37.5%	bl.foot, Ch.mtn, fishing
	Ser86	S→-; S→F	37.5%; 12.5%	bl.foot, Ch.mtn, fishing; Af.lion
	Ser87	S→N	25%	bl.foot, Ch.mtn
	Lys88	K→E; K→V	12.5%; 12.5%	bl.foot; Ch.mtn
	Asp89	D→V	25%	Af.lion, fishing
	Met91	M→I	50%	cougar, lb.lynx, leop.cat, fishing
	Gly92	G→V; G→D	62.5%; 12.5%	cougar, Ch.mtn, lb.lynx, leop.cat, fishing; Af.lion
	Ala94	A→T	12.5%	fishing
	Val95	V→A, V→S	25%; 12.5%	Af.lion, Be.tiger; fishing
	Gln96	Q→E	12.5%	bl.foot