www.sciencemag.org/cgi/content/full/337/6101/1536/DC1

Supplementary Materials for

Specifying and Sustaining Pigmentation Patterns in Domestic and Wild Cats

Christopher B. Kaelin, Xiao Xu, Lewis Z. Hong, Victor A. David, Kelly A. McGowan, Anne Schmidt-Küntzel, Melody E. Roelke, Javier Pino, Joan Pontius, Gregory M. Cooper, Hermogenes Manuel, William F. Swanson, Laurie Marker, Cindy K. Harper, Ann van Dyk, Bisong Yue, James C. Mullikin, Wesley C. Warren, Eduardo Eizirik, Lidia Kos, Stephen J. O'Brien,* Gregory S. Barsh,* Marilyn Menotti-Raymond

*To whom correspondence and requests for materials should be addressed. E-mail: gbarsh@hudsonalpha.org (G.S.B.); lgdchief@gmail.com (S.J.O'B.).

Published 21 September 2012, *Science* **337**, 1536 (2012)
DOI: 10.1126/science.1220893

This PDF file includes:

Supplementary materials:

Materials and Methods

Biological samples

DNA samples and phenotype information from outbred and/or feral cats were collected at five spay/neuter clinics in Northern California. Additional domestic cat DNA samples including breed cats and a research pedigree at the NIH, as well as some cheetah DNA samples, were from a collection maintained at the Laboratory for Genomic Diversity, NCI-Frederick, MD. Samples from the Laboratory of Genomic Diversity were collected in full compliance with specific Federal Fish and Wildlife permits from the Conservation of International Trade in Endangered Species of Wild flora and Fauna: Endangered and Threatened Species, Captive Bred issued to the National Cancer Institute (NCI)-National Institutes of Health (NIH) (S.J.O. principal officer) by the U.S. Fish and Wildlife Services of the Department of the Interior. DNA from the index king cheetah (individual #4 in Fig. S3) was collected from the Wild Cat Conservation and Education Fund in Occidental, California, and studied at Stanford University; DNA samples from the remaining individuals in the captive cheetah pedigree (Fig. S3) were collected at the Ann van Dyk Cheetah Centre from blood samples obtained during routine veterinary examinations, and studied at the University of Pretoria.

For analysis of gene expression in cheetah skin, 4 mm biopsy punches from animals at the Cheetah Conservation Fund (Otjiwarongo, Namibia) were obtained when animals were under general anesthesia during routine veterinary examinations. For histological and gene expression studies of domestic cats, tissue samples from fetal and newborn animals were obtained from the City of Huntsville Animal Shelter from animals that had been euthanized for reasons unrelated to this study.

For studies of *Edn3* expression in the skin of transgenic mice, we made use of a previously described *TRE::Edn3* transgenic line in which expression of *Edn3* requires the presence of a second transgene, *K5::tTA* (*18*). The original description of these animals focused mainly on pigment cell development, documented a very strong effect of the transgene on the accumulation of melanocytes in the fetal dermis, and included one panel showing that the transgene caused darkening of an A^y coat. For analysis of gene expression in these mice, tissues were prepared from animals on a mixed FVB/N, C57BL/6J background; no differences were observed between A^y/-; +/+ animals and A^y/*a*; *Tg.K5-tTA* animals. For analysis of gene expression during mouse development, tissues were prepared from FVB/N animals. All work in mice, cats, and cheetahs was carried out under animal protocols approved by local institutional review boards.

SNP discovery and genome sequences

Genomic DNA isolated from a pedigreed Ta^M/Ta^b cat was amplified using 111 primer sets (Table S3), chosen to amplify potential exons based on comparative mapping (Fig. 1B). Sheared PCR amplicons were sequenced on an Illumina Genome Analyzer IIx. Mapping and Assembly with Qualities (*22*) was used to map sequencing reads to a concatenated reference encompassing all amplicon sequences and to determine heterozygous base positions, as indicated in Table S4.

Primer sets for SNP discovery were designed based on the 1.9x *Felis catus* genome assembly (felCat3, v12.2) (*23*). While this work was underway, a 3x assembly (felCat4) (*24*) and some

information from an ~10x assembly became available (*25*), and was used to design additional primer sets as described in Tables S3 and S4.

Linkage, association and haplotype mapping

Initial linkage studies are described in ref. (*7*); we used information from the 3x genome assembly together with an integrated microsatellite and comparative map (*23*, *26*, *27*) to define the linkage intervals depicted in Fig. 1B. For association mapping in feral cats from Northern California, we initially genotyped 58 SNPs in 8 blotched and 9 mackerel cats, then expanded the sample set to include 16 blotched and 33 mackerel cats for a subset of those SNPs in a second stage (Fig. S1). Initial haplotypes were based on 23 SNPs genotyped in 58 blotched and 19 mackerel cats from the feral cat population in Northern California. Three additional *Tabby* alleles (S59X, T139N, D228N; SNPs "S", "Q", and "P", respectively, Table S3) were discovered by resequencing *Taqpep* exons (Table S4), leading to an expanded set of haplotypes based on 26 SNPs genotyped in 58 blotched, 19 mackerel, and 4 "atypical swirled" cats from the feral population in Northern California (Fig. S2A, S2B, S2C) and 3 blotched and 3 mackerel cats from the NIH colony (Fig. S2D). Genotyping was carried out by capillary-based sequencing; the call rate at each stage (proportion of samples for which an accurate genotype could be inferred) was >95%. Association results (Fig. S1) were evaluated by comparing allele counts from blotched and mackerel cats in a 2x2 contingency table using a Bonferroni-corrected chi-square test (with 1 degree of freedom). Haplotypes were inferred with PHASE v2.1 (*28*). Primer sets and amplicons are described in Tables S3, S4, and S5.

Cheetah genetics

Cheetahs were surveyed for the presence of the *N977Kfs110* allele with 'Exon20c' primer set from Table S4. To test for linkage with the king cheetah phenotype, genotype results for 31 members of a multi-generational captive bred pedigree maintained at the Ann van Dyk Cheetah Center were analyzed with SUPERLINK v1.7 (*29*) under a model of recessive inheritance.

Phylogenetic analysis

To assess the phylogenetic history of *Taqpep* in felids and other mammals, we manually generated an alignment including orthologs from 31 felid species (all of which had identical length proteins), and manually merged this alignment to the *Taqpep* alignment for 23 non-felid mammals (most distant species was platypus) extracted from the 44-way whole-genome vertebrate sequence alignments (http://genome.ucsc.edu). The latter alignment included 23 mammals after removal of non-mammalian vertebrate sequences and any sequence with more than 500 gap characters (the total alignment length was 2,976 nucleotides or 992 codons). The alignment was further projected to a 'felid' frame in which gaps in the felid species were eliminated. A total of three stop codons in two of the 23 non-felid mammalian species were assumed to represent sequence errors and manually changed to gap characters prior to analysis since each of these species exhibited high-quality full-length protein alignments except for the stop codons.

We used MAPP (*12*) to estimate the functional impact of felid-specific amino acid substitutions. Briefly, MAPP quantifies rates of evolution in biochemical terms (*e.g.* amino acid size or polarity) in a group of aligned proteins; the diversity within this alignment is considered to represent the range of

variation compatible with protein function. Functional effects of all substitutions are then estimated on the basis of the phylogenetically weighted biochemical distance between the mutant residue and the aligned sequences. To generate MAPP scores (*12*), we used the felid-projected alignment of the 23 non-felid mammalian *Taqpep* sequences; this allows unbiased estimation of the effects of potentially deleterious, or other change of function, substitutions observed specifically in cats, *i.e.*, if felids were included in MAPP scoring, these substitutions would tend to be considered acceptable and have reduced scores.

Gene expression profiling (EDGE) of cheetah skin

Cheetah skin biopsies were obtained from a black-colored spot and an adjacent yellow-colored background region and preserved in RNAlater (Ambion Life Technologies, Grand Island, NY). Following the isolation of total RNA using a commercial kit (RNeasy Fibrous Tissue Mini kit, Qiagen, Valencia, CA), EDGE libraries were constructed from five pairs of cheetah samples and each library was sequenced on one lane of an Illumina Genome Analyzer IIx. For gene expression profiling, the EDGE protocol and preliminary results obtained with a single animal (that is also included as 1 of the 5 animals presented here) are described in Hong et al. (*13*). In that work, EDGE tags were assigned to genes using the 1.9x assembly (felCat3, v12.2) and a partial transcriptome from the domestic cat. For the work described here, we also used information from an ~10x assembly (*25*).

Differentially expressed genes were identified using an overdispersed Poisson model implemented in the edgeR package (*30*). We carried out a paired analysis by estimating the dispersion parameter using an empirical Bayes method that depends on the overall expression level for each gene. The adjusted gene counts are fit to a negative binomial generalized linear model, and the results are then analyzed with a likelihood ratio test. P values were adjusted for multiple testing using the false discovery rate correction (*31*), and an FDR cutoff of 5% was used to identify differentially expressed genes.

qPCR-based measurements of mRNA levels

Total RNA for qRT-PCR was isolated using Trizol (Invitrogen Life Technologies, Grand Island, NY), purified using RNeasy (Qiagen), and treated with DNaseI (Invitrogen) before reverse transcription with Superscript III (Invitrogen). cDNA was amplified using the LightCycler FastStart DNA Master Plus Sybr Green I System (Roche Diagnostics, Indianapolis, IN). *Bactin* or *Gapdh* were used to compare relative levels of mRNA between different tissues and developmental stages in the mouse (Fig. S6) or between different regions of felid skin (Fig. 4C). Primer sequences are given in Table S9.

In situ hybridization

Digoxigenin-labeled RNA probes were generated from the 3'UTR of mouse and cat *Taqpep* and cat *Edn3* using in vitro transcription (Roche Diagnostics) and a PCR-generated template. Primer sequences are described in Table S9. Prior to embedding, E17.5 mouse embryos, postnatal day 7 mouse dorsal skin, and fetal cat skin was fixed in 4% paraformaldehyde followed by 30% sucrose for 24 hours. Frozen sections (12um) were cut and mounted on Superfrost Plus slides (Fisher

Scientific, Pittsburgh, PA). Sections were fixed with 4% paraformaldehyde for 10 minutes, treated with proteinase K (Sigma, St. Louis, MO), hybridized overnight at 60 degrees, incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche Diagnostics) overnight at 4 degrees and developed 3-6 hours in a buffer containing BCIP/NBT substrate (Roche Diagnostics). Results depicted in Fig. 4C (*Edn3* in cat dermal papilla) were repeated at least 3 times.

Morphometric analysis of cheetah skin

Skin biopsies from black- and yellow-colored areas of 3 individuals were examined, recording 10 high-power fields for each sample, and measuring with ImageJ (*32*) the width of complex follicle clusters, the inter-cluster distance, and the density of pigmented cells in the interfollicular epidermis (Fig. S5A, S5B).

Author contributions

C.B.K. collected the feral cats, carried out the association and haplotype studies, identified the index king cheetah mutation, and coordinated the project. X.X. helped with the association analysis and carried out the sequence analysis of *Taqpep* in breed cats and other felid species as part of a graduate program supervised by B.Y. Gene expression profiling and mouse *Taqpep* expression studies were carried out by L.Z.H. and K.A.M., respectively. V.A.D. carried out the cheetah genotyping and linkage analysis, with support from C.K.H. and A.v.D. The cheetah and leopard skin biopsies were collected by A.S.-K. with the support of L.M., several *F. nigripes* samples were provided by W.F.S, and M.E.R. helped with the collection and analysis of breed cats and cat tissue samples. J. Pontius and H.M. provided bioinformatic and technical support, respectively. G.M.C. carried out the MAPP analysis with input from C.B.K., E.E., and G.S.B. J. Pino and L.K. generated *Edn3* transgenic mice; production of the cat transcriptome and genome assembly was supervised by J.C.M. and W.C.W. Linkage studies for tabby were initiated by E.E. with support from S.J.O., who developed this research initially as leader of the Laboratory for Genomic Diversity. G.S.B. and M.M.-R. coordinated and led the project; G.S.B., C.B.K and M.M.-R. wrote the manuscript with input from V.A.D., A.S.-K., E.E., and S.J.O.

Competing interest statement

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does its mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of manuscript.

Table S1: Survey of genotype/phenotype correlation in feral cats[a]

	Blotched	Mackerel	Atypical Swirled
S59X/W841X	4	0	0
W841X/W841X	54	0	0
S59X/T139N	0	0	1
W841X/T139N	0	7	3
T139N/T139N	0	2	0
Total	58	9	4
S59X/+	0	2	0
W841X/+	0	26	0
T139N/+	0	5	0
+/+	0	9	0
Total	0	42	0

[a] Feral cats from Northern California spay/neuter clinics. The phenotypes of Blotched, Mackerel, or Atypical Swirled are illustrated in Fig. 1 and Fig. S4. Haplotypes for the 58 blotched cats and 19 of the mackerel cats are shown in Fig. S2. An additional *Ta*^{b} allele, D228N, was observed in an NIH colony (Fig. S2).

Table S2. Distribution of *Taqpep* alleles among breed cats

Domestic cat breeds (n)	Geographic origin	Allele Frequency			
		S59X	A/T139N	D228N[a]	W841X
American Curl (4)	Western	0	0	0	0.38
Abyssinian (8)	Western	0	0	0	1
American Shorthair (25)	Western	0.08	0	0	0.82
American Wirehair (8)	Western	0	0	0	0.81
Bengal (16)	Western	0	0.03	0	0.50
Chartreux (11)	Western	0	0	0	0.55
Cornish Rex (20)	Western	0	0	0	0.30
Devon Rex (20)	Western	0	0	0	0.13
Egyptian Mau (14)	Western	0	0.11	0	0.21
Exotic Shorthair (18)	Western	0	0.03	0	0.78
Himalayan (15)	Western	0	0.03	0	0.77
Manx (17)	Western	0	0	0	0.79
Munchkin (15)	Western	0	0.23	0	0.47
Norwegian Forest Cat (12)[b]	Western	0.29	0	0	0.21
Ocicat (16)	Western	0	0.19	0	0.41
Persian (20)	Western	0	0.10	0	0.68
Scottish fold (17)	Western	0	0	0	0.65
Selkirk Rex (16)	Western	0	0	0	0.56
Sphynx (18)	Western	0	0	0	0.11
Turkish Van (8)	Western	0	0	0	0.50
Birman (12)	Eastern[c]	0	0	0	0.71
Bobtail (14)	Eastern	0	0.18	0	0.04
Burmese (12)	Eastern	0	0	0	0
Siamese (15)	Eastern	0	0	0	0.20
Total (351)		0.015	0.04	0	0.48

[a] Mutation observed in NIH animal colony, but not in breed survey.

[b] Among 12 Norwegian Forest Cats, 1 homozygote and 5 heterozygotes or compound heterozygotes were observed for S59X.

[c] Although the Birman breed is said to have an Eastern origin, the breed underwent a severe bottleneck in the 1940s and was outcrossed extensively to Western breeds.

Table S3. Amplicons for *Tabby* candidate region exon sequencing[a]

Amplicon	Size(bp)	Forward primer	Reverse primer
TaExon-Un11-1	460	TCAGGTTCTCTCTGGCCACT	CAGGTGCCCCTAAAGTGATT
TaExon-Un11-2	482	ATCTGCCCACAGCCTTAGTG	TGACAAGGGTCTATTTGGCA
TaExon-Un11-3	2000	TCCAGTTCTCGGTTTTGGTAA	CCGTCATGGGCATAATTTTT
TaExon-Un11-4	470	TTTCAGATACTAACAGTGCCTTTTT	ACCCTTAGCACCCAACAGTG
TaExon-Un11-5	987	ATGGTTCTTTGCTGGTTTGG	AGCATGCACTAGGAGAGTTGC
TaExon-Un11-6	1802	GTTCAATGCCATGTGTGAGG	AGGTGCAGTAATGGATTGGC
TaExon-Un11-7	593	GGCAACACAATTTTAATCATGG	GAAAATGAGAGAATCAGTTTGCTTT
TaExon-Un11-8	602	TACGTGAGTGCCTTGTGAGG	AGCCTGGCCTACAACCCTAA
TaExon-Un11-9	575	GCGTTTGCTGTGATGAAATC	TAGGCGCCCCCTTAAGAATA
TaExon-Un11-10	575	GCGTTTGCTGTGATGAAATC	TAGGCGCCCCCTTAAGAATA
TaExon-Un11-11	617	TGGGACAGTGATAACGATGAAG	TTCAACTGGCCACTGTGTAACT
TaExon-Un11-12	537	TGGGTCATGAATTCTTTTGCT	GACCGCGTGAGACTAGAAGG
TaExon-Un11-13	591	CGTGGCAAACATTATATTGGG	GGGGCTGACAAGAGCATAAG
TaExon-Un11-14	485	TGCACGTTTACACTCAAAGGA	TGCCAAATGTACTTTTTCCTCA
TaExon-Un11-15	628	CCTCCAAACTACCCTCCTCC	GCCCCAACAATGCTTTTATT
TaExon-Un11-16	671	AAATGTGAAATCGGAAGTGTCA	TAATCCAAAAGCACTGGGAA
TaExon-Un11-17	603	CTGCAGAAGACAAATGTGGG	ACGAGCCAAGAACTGCAAAT
TaExon-Un11-18	456	TTCACAGGTTGGGTGTTTTG	TCCAGGAACATTTTTGGAACA
TaExon-Un11-19	608	TCCTGTCTCATTAGGTGAGTCTTTT	GGCGCCCTCTCTTTTAAATAC
TaExon-Un11-20	517	TGGGGGTTAAGAGCTGAAGA	GCTTGCTGAGGACTGATGTG
TaExon-Un11-21	635	CAGATATCTCAGTGTTTGTGCATT	TCACTGGTGCAGGAGTATCG
TaExon-Un11-22	614	TCTTGGGTTTCTCTGACCTACC	CACTGGAAACAAAGTCCGGT
TaExon-Un11-23	544	CCACCAGCAATTTGGGTAAT	AAAAAGCAACAGGCACAAAAA
TaExon-Un11-24	632	TTTTGCACCGTTATGTCGAA	AGGGGAGGGAAAGGATAAGG
TaExon-Un11-25	600	TTCTTCTGCTGGGCAAGTTT	GGCAACAGTTTCTTGAGGTG
TaExon-Un11-26	531	TTCGTGGGCTGATATGTGTC	TGGCAATCATGACAACTCAAA
TaExon-Un11-27	495	CCTCCAAACTACCCTCCTCC	GCTGCTTTACGCCATCTCTC
TaExon-Un11-28	642	CTGTGACAATTTTCCCTGCAT	ATGTCAACCGGAATGAAAGC
TaExon-Un11-29	630	GGCATGTTCTAGAATTGTGACCT	AAACCACATCCCGATTTGTC
TaExon-Un11-30	642	TGGATGCTGAACTAACGCAG	GCGATTTTGGAAGGAAGTGA
TaExon-Un11-31	635	TCCAAGTCTTCTGTGCACCTT	GCAGGCCAAGGATTATCTGA
TaExon-Un11-32	946	GCTGCCCACTCTTATACCCA	GTTCAAACCACATCCCGATT
TaExon-A1-1	648	ACAAAGTCCAAAATCCGTGC	TGTCTCCTTTGTAGATGATAGGTTTG
TaExon-A1-2	547	GGGGGAAAAATGGAACAGAT	TCAGCAAGTTGCCCCTTTAC
TaExon-A1-3	463	GCTGATAATTGAGCTTGGGC	ACACATAGTTGGAGGCAGGG
TaExon-A1-4	496	ATTCAGAGGCTGCTTGCATT	CTAGGATTCCTGCTTGGCTG
TaExon-A1-5	505	GATACATGGGGAAGGAGGGT	CAGAACAGCCATCTCACCAA
TaExon-A1-6	588	AGCCCCTAACTCCCCTACAA	CCAGTGTTGCAAGCCAAAAT
TaExon-A1-7	648	CCTCCAGAAAGATCCCATGA	TACGGCTGGAGATAACCGTC
TaExon-A1-8	608	CCCAATGTCTAATGGCCCTA	ACTTTGGCCGAGTCTTCTCA
TaExon-A1-9	493	CAGCAATGCACTCTGCAAAT	CTCCCAAGACCTGTGACCAT
TaExon-A1-10	492	CCTCTCTGCCGAGAACTTTG	TATGCCCCTTTCAGATCAGC
TaExon-A1-11	541	ATGTTTAGGAGGTTTGGGGC	TGTGGATGGGGGAAAGTAAA
TaExon-A1-12	478	CTTGGAAGGGAATGTGTTGC	TGGCTGAACAAAACGTGGTA
TaExon-A1-13	451	TTTTCAATGCATGATTTTTATCG	CAGGCGCCCCTAGTTTATTT
TaExon-A1-14	645	CCATCATGGGGAAATTTGTT	TTCACAGTGGTCTCCACCAA
TaExon-A1-15	462	GCAGCTGCCAGAATAAAAGG	CCATAAGCGAACCGTGTTTT
TaExon-A1-16	539	ACCCCGTTCACCTCTTTTCT	TTTGTACGCATCTCCTCGTG
TaExon-A1-17	769	CATTTTGACGTGCTCATTGG	CCGGATTCAGGAACCCTATT
TaExon-A1-18	486	GAGAGCCGTTGGTACAGTCC	CACGAAGAGGACAAAACCCT
TaExon-A1-19	573	CTCAAGAACTTCCAGCAGGG	GCATGGGACAGAGTAGGGAA
TaExon-A1-20	640	GCAAGCAAGCTAAATGAGCC	GTGGAAGAGGATTTCCCACA
TaExon-A1-21	450	CCCACTCTGCCCAAGATAAA	AATGTTTGCCCTCTCGTGAC
TaExon-A1-22	1985	AGATTCAACCCTCATGCTGC	GAGTGGGGTGAATGGAAGAA
TaExon-A1-23	1000	GAATTCGTACATGGCGGAGT	ATGGTGAAAAGGGGGATTTC
TaExon-A1-24	643	CACCCAGAATCCTGAGGAAA	GCCCCGAAGATCACCTACTA
TaExon-A1-25	577	ACCTTCATGCCTTCATGGTC	GCATGCAGTACCACATACGG

TaExon-A1-26	587	CCAGAAGCGACTGCATGTTA	CCCAAAGCGCTTAAACTCAG
TaExon-A1-27	464	ATGCCTGGTTTCTCCTTCCT	CGCTGTTCCCTTGATTTGAT
TaExon-A1-28	491	CCTCCAGAAAGATCCCATGA	TTAGAGTCCAGGGGTGGTTG
TaExon-A1-29	936	AGGAAGAAGTGAAGCCCTCC	AAACAACATCGCAAAGGACC
TaExon-A1-30	470	CCTCGCAATCCAAGGTTTTA	TCACGACTCCATGATCCAAA
TaExon-A1-31	480	TGCTCGAACTACCCCTTTGT	CTTGAGAAATCACCCCAGGA
TaExon-A1-32	1665	TTCTGGGGATAGTTGATGGC	GGCCTAAGATTTTGGTGGGT
TaExon-A1-33	890	GAAGGCACAGGTGAGTGAGG	GCGTCCCAGAATGTAGCAGT
TaExon-A1-34	481	AGGGATCTCACCCACTAGTCC	TCATCAACTGAGTATCAACTTCATT
TaExon-A1-35	611	GGGCACCTTTTACTTCACCA	TTGGGCACTTGAATGTACCA
TaExon-A1-36	484	TTCCCTGAAGCTTTAGATTTGC	TCCAGCTGAACAGCTCTCAA
TaExon-A1-37	644	TCCCCACACATACGTTCTCA	TGTTCATTGTCTGGGACCAA
TaExon-A1-38	608	CCCCAAGCCTGTTGTTTTTA	GCCAGAGTTTCCGAAAGTGA
TaExon-A1-39	578	GCGCCATAATAGGGCATTTA	GCTGTCGTGTTAATTGCGAA
TaExon-A1-40	475	GTCAAACCCAGGATGCAGTT	GCTCTTTGGAAGGCGTTATG
TaExon-A1-41	453	TGATTCTCAAAACCTGTCACAAA	ATGAGCCCAAAGGGAGAGTT
TaExon-A1-42	618	AAGATGTTGGCAGCCTGTTT	TTGGCAAAGGATTCTGCTCT
TaExon-A1-43	949	TCCTTGGTGTTTCCATCACA	CCCACGTAGCACAAAACCTT
TaExon-A1-44	590	CCTCTGAACAGGGGACAAAA	GGGAAAAGGTTTAAGCTGGG
TaExon-A1-45	1957	TCGTGACGCTCATCAAAGAC	GGCTACTCTGAACACCCAGC
TaExon-A1-46	603	CAGCACAGGCTCAGATACCA	GGGGATAGGGACAGAAGACC
TaExon-A1-47	540	TTCCAAATACATGGGGCAAT	GCTGGGTTGTTTTCTGTGGT
TaExon-A1-48	485	GCACAGTGCTTAGAGGGGAG	ATTGGTACCCTCCTGCTCCT
TaExon-A1-49	894	AAACTCACAAATCCTCCCCC	CAATGTGGGATGACTCAACG
TaExon-A1-50	620	ACATGGAACCTGAGTCCCAA	GCTGGCCATCATCTGTTTTT
TaExon-A1-51	632	GAAATCGGACCCAGTACGAA	ACCGAATTCCCTAAGGTTGG
TaExon-A1-52	557	CCAATTGGGGATTCAGTTTG	GCAAACCCACATCAATTTCC
TaExon-A1-53	607	CCTGGACCTGTGGAAAGAAA	TCCTTCCGTTTCCCACTAAA
TaExon-A1-54	1508	ACTCTCACCATCCACCCAAG	ACACACACACACACACACGG
TaExon-A1-55	787	TGATGGCAAATCGTCTTCTG	CCTTGAGTTCCTGGTTGCTC
TaExon-A1-56	641	GGGGCATTATGTGTTCATCTG	TGCCTTAGTTCTGTGTTTCCTG
TaExon-A1-57	624	CCATTTGAAAATGCCTCCAT	CTGTCTGTATGGCCAGGGAT
TaExon-A1-58	558	GGTCAGCATCACCTTTTGGT	TGCCTATGGGAAAGTCTGCT
TaExon-A1-59	593	CTTGGCCAGGATAGGAAACA	CCTCTAAGCACTGTGCCCTC
TaExon-A1-60	486	AGCTGATCCCCTGAAGGATT	AAGGACGGATTAGGTCAGCA
TaExon-A1-61	618	CGGATTCAAAGCAAAGTCAA	TCTGCAAACTTTCCTGCTGA
TaExon-A1-62	933	CAAGGACACCATATGGCAAA	CCCTTTGATGAGGGAGAACA
TaExon-A1-63	1000	TGTCATCTCCCACCTCAGTTC	ACCATCCCATACCAATTCCA
TaExon-A1-64	621	CCCCAAGGAAGGTGAGTACA	TGCACTCAAGGTGAGTTTGC
TaExon-A1-65	474	CTGTTCTTTCTCAGGCCGAC	GCCGGGGGTATAATTTAGGA
TaExon-A1-66	493	CAACAATGCCCATTCCTTCT	TGCGCATCCAAAATTATTCA
TaExon-A1-67	613	TCAACTTGGGCCAGATTTTC	CTGCCCTCCCTTCACATTTA
TaExon-A1-68	572	GCTCTTCAGCTGGAAAATGG	TTCAGCGTTCATCCCTTTCT
TaExon-A1-69	479	CACAGTGGGGAAACTGAGGT	ATTTGTGCGCTGACTCAGAA
TaExon-A1-70	549	TCCACTGTATCCATGGAATTTG	AGCGCTGATTCTGAAATGCT
TaExon-A1-71	460	TGCTTCTTAAAACTGCTTGCTG	CTTGTCTCCAGGTTGGGATG
TaExon-A1-72	644	TGTTTGAAGTGGAAAAGGGG	GAGAGCTGCTGGCTTTGTCT
TaExon-A1-73	567	TGTTTGTTACCCAAGGCAGTT	GCGCCCCTTCTTTCTTTTT
TaExon-A1-74	609	GCAGAGGGAACAGCATCAAT	TGTGAAATGGCAGAATTCAAAC
TaExon-A1-75	506	TCACAAGTGAAGGCATTGGA	TTGCTCAGAATAGACGGGCT
TaExon-A1-76	602	CCCACCCTGCAAGAAAATAA	TTACTGGCAGATATGGGGCT
TaExon-A1-77	611	TGCTGGGATTTAACCAAAGG	AATCCTCCAGAGCAGATTCG
TaExon-A1-78	580	AGAGGGCAGCCTGACTTGTA	AGCCTCAATCAAACGCTCAG
TaExon-A1-79	530	CTCTTGCAACAACATGATGGA	CCATCCCATACCAATTCCAA

[a] Primers were designed for 32 exons from chrUn11 and 79 exons from chrA1 based on annotation of the felCat3 assembly

Table S4. Amplicons for *Tabby* region association and haplotype analysis

Amp.	Size	Forward primer	Reverse primer	SNPs[a,b]
TA-1	373	TGAGAAGAGTGGGCCTTTTG	CCTGAGCCTCTATCATCCCA	3 (3062995,3063104,3063115)
TA-2	365	TGGTTTGGTTCTTAGATAAACATCA T	TGGGGCGTTGTGACATATAC	4 (3067830,3067832,3067957,3067991)
TA-3	361	GAATAGCAGAAATTGCATAAGGC	TCAATAAGCAGAAGCATCTTTCA	3 (3074763,3074824,3074911)
TA-4	347	TACGTGAGTGCCTTGTGAGG	AGGTGCAGTAATGGATTGGC	2 (3077707,3077733)
TA-5	393	GCAGTGATGGAGAGACCGTT	TTCACATGACTCCTCATTCCA	3 (3098108,3098291,3098311)
TA-6	397	TGCAATTCTCCGCCATAAAG	CTGCAAATTAGACACAGAAGGC	6 (3099360,3099444,3099501,3099534,3099537,3099584)
TA-7	397	AGATCCAAACACCATCTGGC	TGACAACTCAAAGCACACCA	1 (3100437)
TA-8	358	TTGCTCAAGCCATGATGTTC	TCTTCAGCTCTTAACCCCCA	1 (3104664)
TA-9	345	AGCTGTGCAAGGTACAACCC	GCTTGCTGAGGACTGATGTG	1 (3105186)
TA-10	374	GAAGGGAGGAAGTTTTCCCA	GAATTCATCCCCGAAAGGTT	1 (120845010)
TA-11	277	GGTGGAAATGGCATCTTTTG	TTTGTACGCATCTCCTCGTG	2 (120853237,120853241)
TA-12	368	GGCTGGAGAAACCAAATTCA	CGTGCAGCTTGGAAAACATA	2 (120858757,120858904)
TA-13	281	CTCTATGGAATGAGGAGGCG	GGGTGCGTTTTGATGAAGTT	1 (120861377)
TA-14	398	GCAAGCAAGCTAAATGAGCC	TTCGTACTGGGTCCGATTTC	1 (120875097)
TA-15	261	CGCCAGTAAGGAGTGAGAGG	AGAAAAGGGGCTGAGAAACC	1 (120889838)
TA-16	267	TCACTCCCAGCTGTAACACG	ATGAGCCCAAAGGGAGAGTT	*2 (A:121238539,B:121238572)*
TA-17	336	TTTCTTTTGCCAGACTGCCT	TGAGTATAATTCAAGGGATCTGTCTG	*1 (C:121262789)*
TA-18	336	AGGAGCCCCAAAAGCATTAT	TGTCCTAGGGAACACAGGCT	*1 (D:121298806)*
TA-19	271	CCCCAAGCCTGTTGTTTTTA	TCAATAGGGGCTGTACGCTT	*1 (E:121344960)*
TA-20	492	CCTCTCTGCCGAGAACTTTG	TATGCCCCTTTCAGATCAGC	*2 (F:121355037,G:121355078)*
TA-21	364	GCTGATAATTGAGCTTGGGC	TTTTTGAAACTGCGTGTTGG	*2 (H:121356067,I:121356070)*
TA-22	370	TAGTAGGTGATCTTCGGGGC	TCTTAGGGAGCATCTTTGGC	*1 (J:121370158)*
TA-23	367	ACCCTTGCAGATCCAGAGAA	TCCGTGTTCAACCAGAATGA	*2 (K,L)*
TA-24	363	TCTGAAATGCACCCACTCAA	GCATTTTGAAAGCATTTGGA	*1 (M)*
TA-25	349	GAATCAGGCTGGCAAACATTA	CCATTTCATTCTACTTTGGTAGCC	*1 (N)*
TA-26	344	CCTCTCAAGGAAGCTACAAGGT	AACCAGATCGTGTGTGTTTCTG	*1 (O)*
TA-27	457	CTCGTGCCTCTGCACTATGA	ACTGGGACGCAGGTAGCA	*1 (P)*
TA-28	548	CCTTCCAGCTCTGGCTTCTA	ATGTCGAAAGCGAACCACA	*3 (Q,R,S)*
TA-29		TCATCAGGTTCAGGGTTCTTG	AAAAAGAAAATAAATATCTGAGTCC A	*1 (T)*
TA-30	311	AAGGGACCGGATGAAAGACT	AGCACCTTTCTGTTTGCCTG	*2 (U:121510763,V:121510796)*
TA-31	311	GACCCCTTGGGACTGTATTACT	CCCTGTTCAGAGGGACAGAA	*3 (W:12119320,X:12119332,Y:12119369)*
TA-32	272	TGAAGAAGCCTCTCAGCTGTC	GTGAGTTTTCCTTACGGCCA	*1 (Z:121528106)*
TA-33	346	GCGTTAGATAGGCAGCAAGG	CCAATCAAGGAATCAGGACAG	1 (122232786)
TA-34	319	AAACCCATATTAGATCCACCTGAA	TTCAGCGTTCATCCCTTTCT	2 (122272066,122272069)
TA-35	367	TCCTTGGTGTTTCCATCACA	ACATTCAGGGCAGGATGAAC	1 (122299065)

[a] Chromosome assignment and SNP position are based on *Felis catus* assembly felCat3, v12.2 (coordinates are not provided for amplicons that are not in the assembly). Amplicons TA-1 – TA-9 are from an unassigned contig (Un11); amplicons TA-10 – TA-35 are from chromosome A1. Size is given in bp.

[b] Italicized SNPs were used for haplotype analysis. Letter designations correspond to the SNP column labels in Figure 1c and fig. S2,S3, and S4.

Table S5. Amplicons for *Taqpep* sequencing in domestic cats and cheetahs

Amplicon [a]	Size(bp)	Forward primer	Reverse Primer
Exon1a[b]	548	CCTTCCAGCTCTGGCTTCTA	ATGTCGAAAGCGAACCACA
Exon1b[b]	457	CTCGTGCCTCTGCACTATGA	ACTGGGACGCAGGTAGCA
Exon1c	655	AAACATCCTCGTCGGAAGTG	ATGTCGAAAGCGAACCACA
Exon2	390	ACAAGCCATCATCCCTCAAT	ACCAACACACGGCCTAGAAA
Exon3	313	CTTAGGGGTGCAGAAATCCA	ACCTGTGGCCAGTATGAAGG
Exon4	396	GGCTTTGACTCGAGAGCATC	TGTGTTTGGGAAGGTGTCAA
Exon5	383	TTTTCTGGATTCTGTCTTCGG	CCTTGGTAGAGCTGCTGGAG
Exon6	387	TCAGAGAAATGCACGACTGC	CACACTCGAAACAATGAAATGC
Exon7	363	TCTGAAATGCACCCACTCAA	GCATTTTGAAAGCATTTGGA
Exon8	337	GCAATGAGAGAAAAGCCCAG	CCATTTGAAAATGCCTCCAT
Exon9	308	TGCACTCAAGGTGAGTTTGC	TAGTAGGTGATCTTCGGGGC
Exon10a	333	GCCCCGAAGATCACCTACTA	CCCCAAGGAAGGTGAGTACA
Exon10b	272	TGCTCTTGTGATGATTTTCCA	CAGGCTGCTTTCCATTTGAT
Exon10c	130	AAAGCATAATGGACCGTTGG	TTGTGGGTTAGGAGAGTCTGA
Exon11	367	ACCCTTGCAGATCCAGAGAA	TCCGTGTTCAACCAGAATGA
Exon12	379	TTTTTGTTGGATTTACTGGGTTT	TCCCTGTCTCTCTACCCTTCC
Exon13	381	AGCGCTGATTCTGAAATGCT	GTTAAGCGTCCGACTTCAGC
Exon14	396	TGTTGAGATCAGGCATCGAA	AGCCGTCCCTAAGAAAACCA
Exon15	301	CCAGTGTTGCAAGCCAAAAT	CATGTAGCTCACCAGTCCCA
Exon16	377	TTATTTGGTGCCTGAAAGCC	TGGTGTTGAAACAGGACGAA
Exon17a	385	CCAGGCACCCTGCTAACTAA	TTAACTGACTGAGCCACCCA
Exon17b[b]	220	CTCTCTGCCGAGAACTTTGG	GCATTGCACCTTCTACCTTACA
Exon18	399	GCGCTGACTCAGAACCCTAA	AGCGACCTTCTCTTCCACAA
Exon19	271	TCAATAGGGGCTGTACGCTT	CCCCAAGCCTGTTGTTTTTA
Exon20a	1979	TTGGCCTTCTTCTGGCTAAA	TGGAAGTGGGACTGTCATCA
Exon20b	319	GTTGGAAGAGCACCAGAAGC	CAAAACCCTCAGCCATCACT
Exon20c[b]	688	TTGGCCTTCTTCTGGCTAAA	TGCTTTCCTTGGCTTTTCTG

[a] Some exons were amplified with multiple primer sets due to exon size, amplification difficulties, or sequence variants within primer binding sites.

[b] Primer sets used for amplification and sequencing of the cat and cheetah *Taqpep* mutations are: Exon1a (S59X and T139N), Exon1b (D228N), Exon17b (W841X), and Exon20c (N977Kfs110).

Table S6. Genotype and phenotype frequencies in feral cats[a]

	Blotched[b]	Mackerel[b]	
		Observed	Expected
$Ta^M/\ Ta^M$	0	16	16.6
$Ta^b/\ Ta^M$	0	36	35.4
$Ta^b/\ Ta^b$	31	0	0
Total	31	52(85)	52

[a] Prior phenotype-based studies have reached conflicting conclusions with regard to potential effects of Ta^b on fitness (*33-35*). The genotype distribution data shown here, based on feral cats from Northern California, are consistent with Hardy-Weinberg expectation.

[b] Among 311 individuals, 116 had distinct tabby markings that allowed them to be classified as blotched (n=31) or mackerel (n=85). Assuming Hardy-Weinberg equilibrium and complete penetrance of the Ta^b allele, this distribution (31 Ta^b/Ta^b and 85 Ta^b/Ta^b or Ta^M/Ta^b) predicts a Ta^b allele frequency of 0.517. Of the mackerel cats, 52 were genotyped (Ta^b refers to the S59X (n=2) or the W841X (n=34) mutations). This population partially overlaps the individuals reported in Table S1.

Table S7. Genes upregulated in black spot areas of cheetah skin[a]

Gene	Fold Change	Pathway[b]	P value	FDR
SILV	7.33	Melanogenesis	7.33E-41	1.03E-36
JAKMIP1	28.1		1.10E-34	7.71E-31
TYR	4.92	Melanogenesis	8.22E-23	3.84E-19
TRPM1	14.5	Melanogenesis	4.10E-20	1.44E-16
ENSFCAG00000010136	3.14		5.89E-15	1.38E-11
HBB	2.94		6.86E-13	1.20E-09
NDUFA2	2.99		1.14E-12	1.78E-09
DCT	3.19	Melanogenesis	4.19E-11	5.33E-08
LIMD2	5.21		6.35E-10	6.36E-07
CA8	7.28		3.92E-09	3.66E-06
RPL36AL	2.6		1.11E-08	9.71E-06
TYRP1	3.08	Melanogenesis	2.21E-08	1.72E-05
DYNC1I1	2.55		5.79E-08	4.06E-05
PLXNC1	2.48		8.40E-08	5.61E-05
RPL26L1	2.06		2.13E-07	1.36E-04
CORO1A	1.97		2.62E-07	1.60E-04
EDN3	4.87	Paracrine signaling	3.59E-07	2.10E-04
LTB	3.21		1.37E-06	7.39E-04
SPI1	2.39		1.36E-06	7.39E-04
OSR1	2.32		1.62E-06	8.41E-04
LRRC33	2.38		3.66E-06	1.83E-03
RAB3IL1	1.91		3.82E-06	1.85E-03
ST6GALNAC3	2.75		4.00E-06	1.87E-03
TMEM200B	4.4		7.66E-06	3.32E-03
FOLR2	1.8		7.82E-06	3.32E-03
CLDN5	1.82		1.11E-05	4.57E-03
C16orf38	2.44		1.44E-05	5.77E-03
MLANA	3.43	Melanogenesis	1.55E-05	5.87E-03
NEDD8	2.9		1.51E-05	5.87E-03
SAA3P	1.76		1.69E-05	6.22E-03
SLC24A5	2.72	Melanogenesis	1.74E-05	6.24E-03
DOK1	2.0		2.05E-05	7.00E-03
C1QTNF5	1.72		2.01E-05	7.00E-03
CHTF18	1.85		2.94E-05	9.57E-03
SVEP1	1.71		3.40E-05	1.08E-02
SLC43A1	1.72		4.30E-05	1.31E-02
SERPING1	1.7		4.30E-05	1.31E-02
TRAFD1	1.75		4.96E-05	1.45E-02
STAT4	2.64		6.15E-05	1.72E-02
CD248	1.68		6.36E-05	1.75E-02
SEPT1	2.65		7.22E-05	1.87E-02
MFAP2	1.76		7.22E-05	1.87E-02

LOC729085	1.99	8.87E-05	2.26E-02
ZIC1	3.62	9.43E-05	2.36E-02
CNNM1	3.7	1.02E-04	2.50E-02
CD209	2.72	1.10E-04	2.66E-02
CCL14	1.65	1.18E-04	2.81E-02
ENSFCAG00000002482	6.45	1.24E-04	2.85E-02
COL6A6	6.05	1.26E-04	2.85E-02
CLEC10A	1.67	1.25E-04	2.85E-02
CLEC1A	2.26	1.34E-04	2.90E-02
BDKRB2	1.72	1.34E-04	2.90E-02
LRRN4CL	1.66	1.32E-04	2.90E-02
RBM19	2.4	2.24E-04	4.61E-02
HIST2H2AA4	3.84	2.35E-04	4.72E-02
NAPSA	1.71	2.39E-04	4.72E-02
PLBD2	1.6	2.36E-04	4.72E-02
MUTYH	1.72	2.54E-04	4.94E-02
TPRA1	3.15	2.61E-04	4.98E-02
TSPAN4	1.61	2.63E-04	4.98E-02

[a] Based on a digital gene expression approach (EDGE) as described in Methods. Genes (FDR < 0.05) are listed in order of increasing P values for significance.
[b] Among 60 genes upregulated in skin from the black spot compared to the yellow inter-spot area, seven encode genes involved in melanogenesis and one encodes a paracrine factor that are candidates for eliciting localized differences in hair color.

Table S8. Genes upregulated in yellow inter-spot areas of cheetah skin[a]

Gene	Fold Change	Pathway[b]	P value	FDR
ENSFCAG00000004053	4.52		1.40E-15	3.93E-12
ENSFCAG00000015326	2.83		1.72E-14	3.44E-11
ENSFCAG00000004061	2.38	KRTAP19-7	1.87E-11	2.62E-08
ENSFCAG00000015327	2.32	KRTAP21-1	8.46E-11	9.87E-08
ENSFCAG00000015329	2.23	KRTAP21-2	3.91E-10	4.22E-07
ENSFCAG00000002173	2.11	KRTAP20-2	1.45E-08	1.20E-05
ENSFCAG00000004049	2.08	KRTAP20-1	3.17E-08	2.34E-05
SPINK6	2.03		6.24E-06	2.82E-03
ENSFCAG00000006428	1.72	KRTAP1-3	2.41E-05	8.05E-03
PCP4	1.81		4.82E-05	1.44E-02
MT1L	1.73		5.69E-05	1.63E-02
TMCO4	1.82		7.04E-05	1.87E-02
SDHB	1.62		1.69E-04	3.59E-02
LYG1	2.44		2.04E-04	4.27E-02

[a] Based on a digital gene expression approach (EDGE) as described in Methods. Genes (FDR < 0.05) are listed in order of increasing P values.

[b] Among 14 genes upregulated in skin from the yellow inter-spot compared to the black spot area, six encode keratin-associated proteins based on sequence similarity to the indicated human proteins.

Table S9. Amplicons used for expression studies

Amplicon	Species	Forward primer	Reverse Primer
Pmel[a]	Mouse	GCACCCAACTTGTTGTTCCT	AGAGATGCAAGGACCACAGC
Slc24a5[a]	Mouse	ACATCCTAGTTTGGATGGTCAC	CTGGTATGCTTGTCCCTGCT
Mlana[a]	Mouse	GCACAGACGCTCCTATGTCA	AGCCGATAAGCAGAGCAATC
Tyr[a]	Mouse	AAATCATCAAGCCCAAGAGC	TGCCCCTGACACTATCACAC
Trpm1[a]	Mouse	GCCTCCTAGCTTTCACATGC	AAGGAGGGGAGGACAGACAT
Edn3[a]	Mouse	AGGCCTGTGCACACTTCTGT	CAGTCTCCCGCATCTCTTCT
Dct[a]	Mouse	GCGTGCTGAACAAGGAATG	CCAGGGTCTGGTGTCTGTTT
Tyrp1[a]	Mouse	TGCTCCAGACAATCTGGGATA	AACGCAGCCACTACAGCAAT
Slc7a11[a]	Mouse	GGCACCTTTGTTCTGGTGAT	ACGTGAGGAACGCAGAGAAC
Bact[a]	Mouse	CGAGCACAGCTTCTTTGCAG	GCAGCGATATCGTCATCCAT
Taqpep[b]	Mouse	CTGAAGTTGGCCGGTACATT	CCCTGGCAATTCTCTTCTTG
Edn3[c]	Cat	ATCTCTGGGAGGCCTCAGTT	GTCCTCAACTCCTGCAAAGC
Edn3[d]	Cat	GGGGAAATTTAAGGTGGTGAA	TCCGGGTGATAGGTACTCCTT
Gapdh[d]	Cat	AAGGTCATCCCAGAGCTGAAT	AGATCCACGACGGACACATT
Pmel[d]	Cat	AGGGACCTACTGCCTCAATGT	AAGCACCATAGCCATCAACAC
Dct[d]	Cat	GAGCCTGCATAACTTGGTTCA	ATCCACAGGAGGATTGGATCT
Taqpep[d]	Cat	TGGCAGCGTTACAAGATGAC	ACTTCAGATTCCGCCACAAC
Mitf[d]	Cat	CTATAGCGTCCCCACGAAAA	TTTCTTCCATGCTCATGCTG
Slc7a11[d]	Cat	GCCCATTACCAGCTTTCGTA	CACGGCTGTAATGAGCTTGA

[a] For studies of mRNA expression by qPCR in *Tg.TRE-Edn3* mice (Fig. 4D)

[b] For studies of *Taqpep* mRNA expression by qPCR in mice (Fig. S6)

[c] For studies of *Edn3* mRNA expression by in situ hybridization in cats (Fig. 4C)

[d] For studies of mRNA expression by qPCR in cats, cheetahs, and leopards (Fig. 4C, S5C)

Supplemental Figure 1

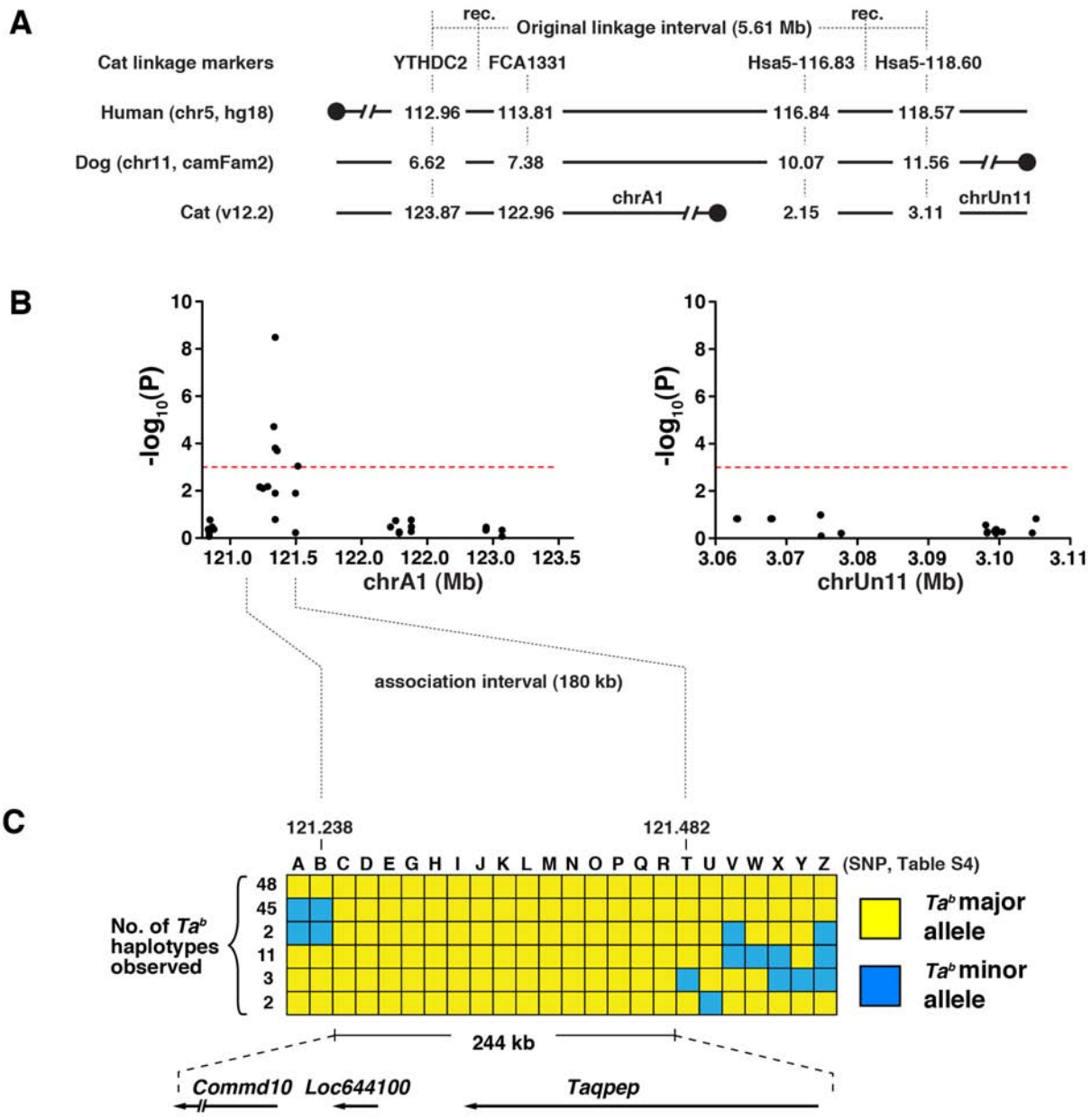


Fig S1. Genetic mapping of the *Tabby* gene. (A) Recombination (rec.) breakpoints for the *Tabby* linkage region lie on chromosome A1 and an unassigned contig (chrUn11), and correspond to a candidate interval of ~ 5 Mb in the dog (chr11: 6.62-11.56 Mb) and human (chr5: 112.96-118.60 Mb) genomes. (B) Significance of genotype-phenotype association, plotted as $-\log_{10}(P)$ from a chi-square test of allele counts is shown as a function of distance along chrA1 or chrUn11 for 58 SNPs (Table S4) in Ta^b/Ta^b (n = 8-16) compared to $Ta^M/-$ (n = 9-32) random-bred animals. The dashed red line indicates a Bonferroni-corrected 5% significance level. (C) Haplotype analysis (Table S4) narrows the interval to a 244 kb region (chrA1:121238572-121482501) containing 3 genes. The 111 Ta^b chromosomes summarized here do not include 5 singletons; all Ta^b chromosomes are shown separately in Fig. S2.

Supplemental Figure 2

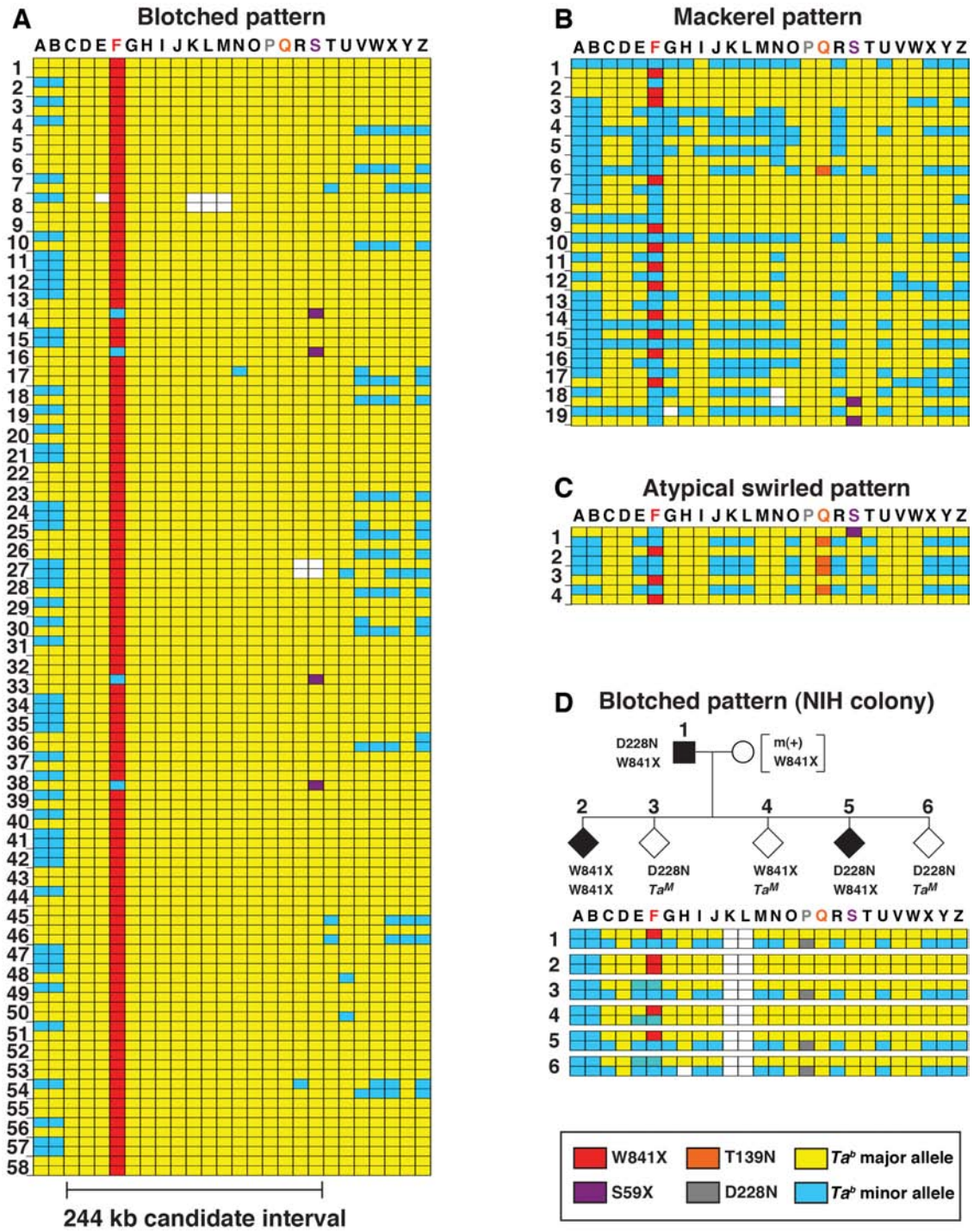


Fig. S2. Haplotypes for tabby patterns. Phenotypes are shown in Fig. 1 (blotched, mackerel) and Fig. S4D (atypical swirled). The S59X, T139N, and W841X alleles were observed in feral cats from a Northern California population (A, B, C) and also in breed cats (Table S2); the D228N mutation was observed only in animals from an NIH colony (D). SNP positions and genotyping information are given in Table S4.

Supplemental Figure 3

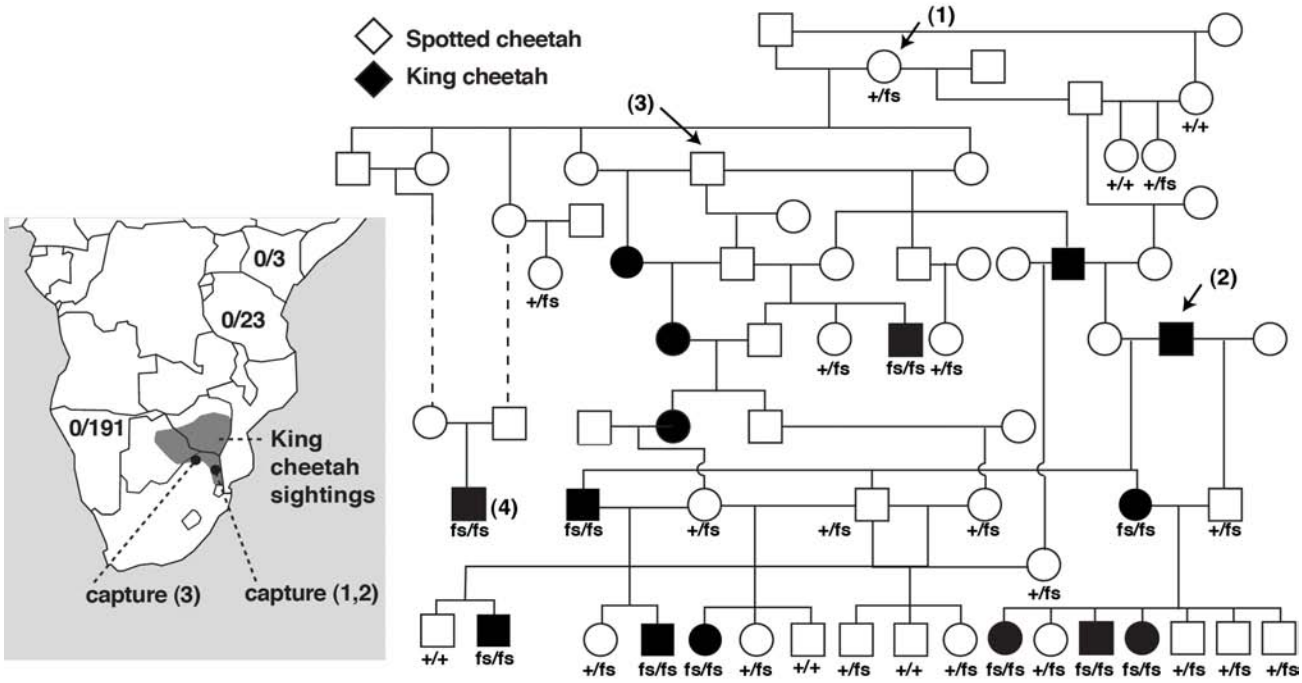
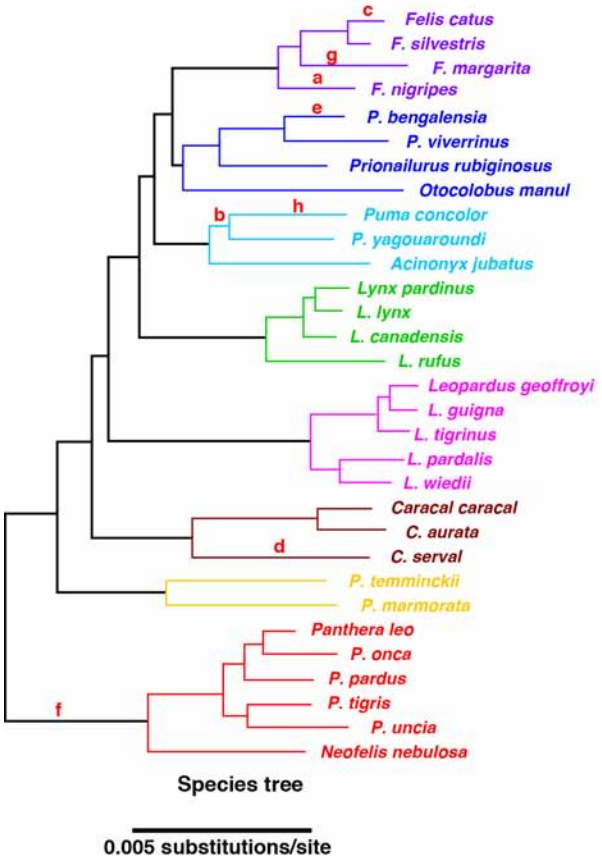


Fig. S3. (Left) King cheetah sightings have been restricted to a small (grey-colored) region that includes the Northeast corner of South Africa and parts of Botswana and Zimbawe. (Right) The K977Nfs110 mutation is completely linked to the king cheetah pattern (LOD = 5.7, $\theta = 0$) under a model of autosomal recessive inheritance with complete penetrance. Genotype results are shown for 32 captive animals from the DeWildt pedigree (non-mutant, +, or mutant, fs), and reveal that the frameshift mutation was introduced into the DeWildt pedigree by 3 captured animals (1-3). The proband animal from Northern California (4) is also derived from the DeWildt pedigree; dashed lines indicate 5 generations in which no king cheetahs were observed.

Supplemental Figure 4



B

Position	82	110	139	228	282	603	817	880	950	975	989
Human	T	L	T	D	Q	R	E	A	F	L	R
Rhesus	T	L	T	D	Q	Q	E	A	F	L	R
Mouse	G	L	A	D	Q	Q	E	A	F	L	R
Guinea Pig	P	L	T	D	Q	Q	K	A	F	L	R
Horse	A	L	I	D	Q	Q	E	A	F	L	R
Dog	T	L	T	D	E	R	E	A	F	L	R
Microbat	T	L	T	D	Q	Q	E	A	F	L	R
Felid Mut.	K	V	N	N	E	S	G	V	V	V	W
Location	a	b	c	c	d	e	f	g	a	f	h
MAPP sc.	25	26	11	14	12	13	11	14	18	26	12
P value	***	***	*	**	*	**	*	**	**	***	*

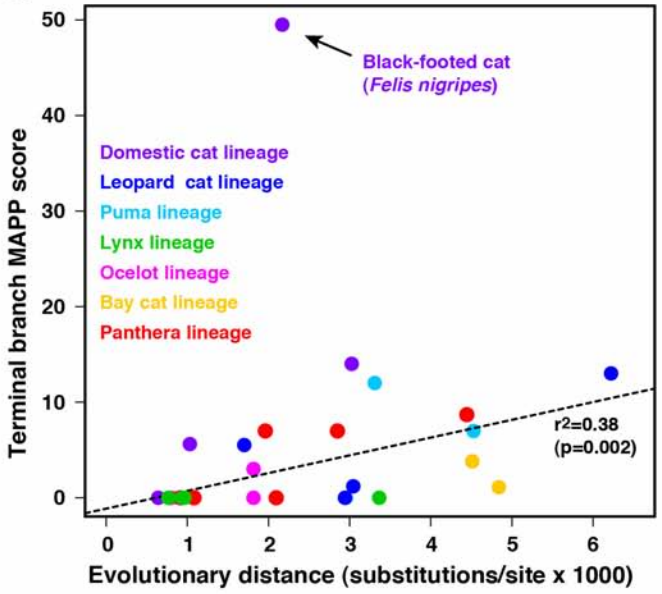


Fig S4. *Taqpep* variation during felid evolution. (A) Phylogeny of 31 felid species for which *Taqpep* sequence was determined, together with the inferred location of selected *Taqpep* substitutions depicted in panel (B). Topology and branch lengths for the tree are based on Johnson et al. (*36*) (B), Non-synonymous substitutions predicted to have a significant impact on protein function (MAPP score >10). Their assignment to branch locations on the felid tree is based on a maximum likelihood analysis in the context of a known phylogeny (A) as described in Methods. *, **, and *** indicate *P* values $< 5 \times 10^{-3}$, $< 5 \times 10^{-4}$, and $< 5 \times 10^{-5}$, respectively. (C) The potential impact of substitutions in each of 31 terminal branches (determined by the sum of MAPP scores for that branch) plotted as a function of terminal branch length based on (A). Species are organized into 7 main lineages according to Johnson et al. The regression line is based on all points except *F. nigripes* (the black-footed cat). (D) Pattern phenotype of *F. nigripes*, which resembles the atypical swirled pattern observed in domestic cats that carry the T139N allele (Fig. S2). Nine of nine *F. nigripes* individuals were fixed for 4 species-specific variants (T82K, H87P, E488K, F950V). Photographs were available for 4 of the 9 individuals that were sequenced (right-hand four panels); DNA was not available for the individual depicted in the upper left panel (from Pierre de Chabannes for www.photozoo.org).

Supplemental Figure 5

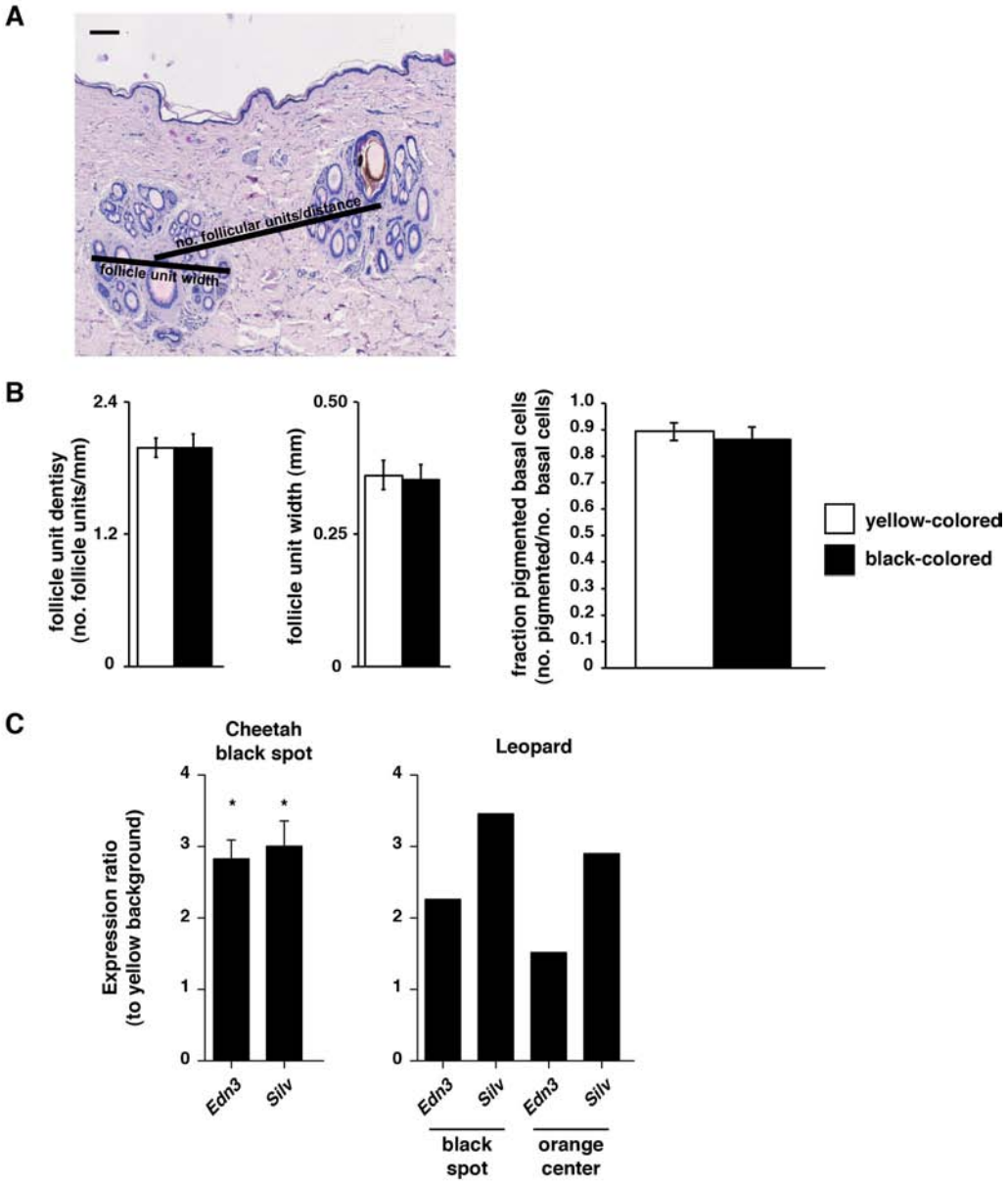


Fig. S5. Pattern characteristics of wild cat skin. (A) Hematoxylin and eosin-stained section of cheetah skin that includes a cross-section of complex follicle clusters from black (left) and yellow (right) colored areas. Scale bar: 100 uM. (B) Follicle density, follicle width, and the density of interfollicular epidermal cells (mean ± standard error) are indistinguishable between black- and yellow-colored areas. (C) Ratio of *Edn3* and *Silv* mRNA levels in cheetah (n=4) and leopard (n=1) skin regions, compared to the inter-spot yellow background areas. * $P<0.05$ (spot vs. background, two-tailed t test). The cheetah samples used here are distinct from those used for gene expression profiling (Fig. 5B).

Supplemental Figure 6

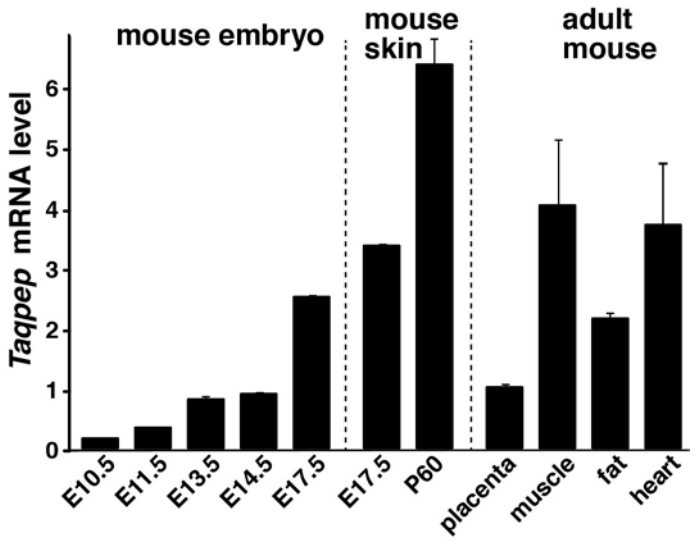


Fig. S6. Relative *Taqpep* mRNA levels in the laboratory mouse, measured by qRT-PCR from whole embryos, from embryonic (E17.5) and adult (P60) skin, and from other tissues. Results shown represent the mean ± standard error of three animals.

Supplemental Figure 7

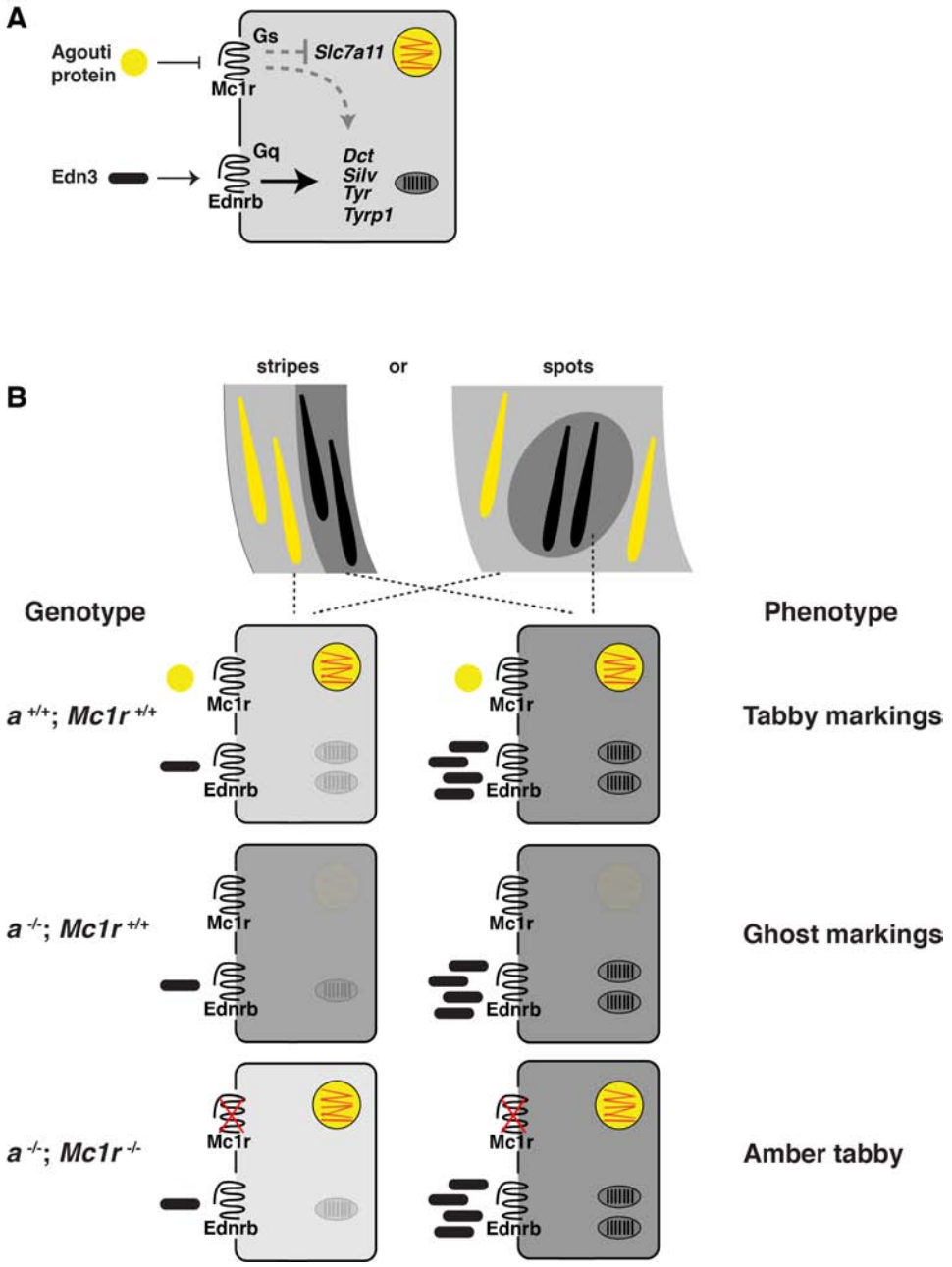


Fig. S7. Effects and interactions of endothelin and melanocortin signaling in cats. (A) The ability of *Edn3* to engage downstream eumelanic components is likely due to overlap between targets of Gs and Gq, the G proteins utilized by the Mc1r and Ednrb, respectively. The cartoon represents a single melanocyte. Red/yellow pheomelanosomes are produced when expression of *Slc7a11* is increased, and expression of *Dct*, *Silv*, *Tyr*, and *Tyrp1* are decreased; conversely, black/brown eumelanosomes are produced when expression of *Slc7a11* is decreased, and expression of *Dct*, *Silv*, *Tyr*, and *Tyrp1* are increased. (B) Interactions between *Agouti* and *Mc1r* in the context of tabby markings. Amber tabby cats exhibit strong tabby markings as kittens that fade during maturation (*5*). Diagrams are based on the expression results reported here (Fig. 4) and on ref. (*5*, *6*, *14*, *15*).

References

1. T. D. Lomax, R. Robinson, Tabby pattern alleles of the domestic cat. *J. Hered.* **79**, 21 (1988). Medline

2. A. G. Searle, *Comparative Genetics of Coat Color in Mammals* (Academic Press, New York, 1968).

3. S. E. Millar, M. W. Miller, M. E. Stevens, G. S. Barsh, Expression and transgenic studies of the mouse agouti gene provide insight into the mechanisms by which mammalian coat color patterns are generated. *Development* **121**, 3223 (1995). Medline

4. I. J. Jackson, Molecular and developmental genetics of mouse coat color. *Annu. Rev. Genet.* **28**, 189 (1994). doi:10.1146/annurev.ge.28.120194.001201 Medline

5. M. Peterschmitt, F. Grain, B. Arnaud, G. Deléage, V. Lambert, Mutation in the melanocortin 1 receptor is associated with amber colour in the Norwegian Forest Cat. *Anim. Genet.* **40**, 547 (2009). doi:10.1111/j.1365-2052.2009.01864.x Medline

6. E. Eizirik *et al*., Molecular genetics and evolution of melanism in the cat family. *Curr. Biol.* **13**, 448 (2003). doi:10.1016/S0960-9822(03)00128-3 Medline

7. E. Eizirik *et al*., Defining and mapping mammalian coat pattern genes: Multiple genomic regions implicated in domestic cat stripes and spots. *Genetics* **184**, 267 (2010). doi:10.1534/genetics.109.109629 Medline

8. C. A. Driscoll *et al*., The Near Eastern origin of cat domestication. *Science* **317**, 519 (2007). doi:10.1126/science.1139518 Medline

9. M. Maruyama *et al*., Laeverin/aminopeptidase Q, a novel bestatin-sensitive leucine aminopeptidase belonging to the M1 family of aminopeptidases. *J. Biol. Chem.* **282**, 20088 (2007). doi:10.1074/jbc.M702650200 Medline

10. R. I. Pocock, *Proc. Zool. Soc. London* **97**, 245 (1927).

11. R. J. van Aarde, A. van Dyk, *J. Zool.* **209**, 573 (1986).

12. E. A. Stone, A. Sidow, Physicochemical constraint violation by missense substitutions mediates impairment of protein function and disease severity. *Genome Res.* **15**, 978 (2005). doi:10.1101/gr.3804205 Medline

13. L. Z. Hong, J. Li, A. Schmidt-Küntzel, W. C. Warren, G. S. Barsh, Digital gene expression for non-model organisms. *Genome Res.* **21**, 1905 (2011). doi:10.1101/gr.122135.111 Medline

14. C. S. April, G. S. Barsh, Skin layer-specific transcriptional profiles in normal and recessive yellow (Mc1re/Mc1re) mice. *Pigment Cell Res.* **19**, 194 (2006). doi:10.1111/j.1600-0749.2006.00305.x Medline

15. T. Kobayashi *et al*., Modulation of melanogenic protein expression during the switch from eu- to pheomelanogenesis. *J. Cell Sci.* **108**, 2301 (1995). Medline

16. C. D. Van Raamsdonk, K. R. Fitch, H. Fuchs, M. H. de Angelis, G. S. Barsh, Effects of G-protein mutations on skin color. *Nat. Genet.* **36**, 961 (2004). doi:10.1038/ng1412 Medline

17. C. D. Van Raamsdonk, G. S. Barsh, K. Wakamatsu, S. Ito, *Pigment Cell Melanoma Res.* **22**, 819 (2009).

18. R. J. Garcia *et al*., Endothelin 3 induces skin pigmentation in a keratin-driven inducible mouse model. *J. Invest. Dermatol.* **128**, 131 (2008). doi:10.1038/sj.jid.5700948 Medline

19. A. Nakamasu, G. Takahashi, A. Kanbe, S. Kondo, Interactions between zebrafish pigment cells responsible for the generation of Turing patterns. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 8429 (2009). doi:10.1073/pnas.0808622106 Medline

20. M. Iwashita *et al*., Pigment pattern in jaguar/obelix zebrafish is caused by a Kir7.1 mutation: Implications for the regulation of melanosome movement. *PLoS Genet.* **2**, e197 (2006). doi:10.1371/journal.pgen.0020197 Medline

21. M. Watanabe *et al*., Spot pattern of leopard Danio is caused by mutation in the zebrafish connexin41.8 gene. *EMBO Rep.* **7**, 893 (2006). doi:10.1038/sj.embor.7400757 Medline

22. H. Li, J. Ruan, R. Durbin, Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res.* **18**, 1851 (2008). doi:10.1101/gr.078212.108 Medline

23. J. U. Pontius *et al*.; Agencourt Sequencing Team; NISC Comparative Sequencing Program, Initial sequence and comparative analysis of the cat genome. *Genome Res.* **17**, 1675 (2007). doi:10.1101/gr.6380007 Medline

24. J. C. Mullikin *et al*.; NISC Comparative Sequencing Program, Light whole genome sequence for SNP discovery across domestic cat breeds. *BMC Genomics* **11**, 406 (2010). doi:10.1186/1471-2164-11-406 Medline

25. The Genome Institute at Washington University, http://genome.wustl.edu/genomes/view/felis_catus/ (2011).

26. B. W. Davis *et al*., A high-resolution cat radiation hybrid and integrated FISH mapping resource for phylogenomic studies across Felidae. *Genomics* **93**, 299 (2009). doi:10.1016/j.ygeno.2008.09.010 Medline

27. M. Menotti-Raymond *et al*., An autosomal genetic linkage map of the domestic cat, *Felis silvestris catus*. *Genomics* **93**, 305 (2009). doi:10.1016/j.ygeno.2008.11.004 Medline

28. M. Stephens, N. J. Smith, P. Donnelly, A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* **68**, 978 (2001). doi:10.1086/319501 Medline

29. M. Fishelson, D. Geiger, Exact genetic linkage computations for general pedigrees. *Bioinformatics* **18** (suppl. 1), S189 (2002). doi:10.1093/bioinformatics/18.suppl_1.S189 Medline

30. M. D. Robinson, D. J. McCarthy, G. K. Smyth, edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139 (2010). doi:10.1093/bioinformatics/btp616 Medline

31. J. D. Storey, The positive false discovery rate: A Bayesian interpretation and the q -value. *Ann. Stat.* **31**, 2013 (2003). doi:10.1214/aos/1074290335

32. M. D. Abramoff, P. J. Magalhães, S. J. Ram, *Biophotonics International* **11**, 36 (2004).

33. J. L. Dards, R. Robinson, Gene frequencies in a population of feral cats in Portsmouth naval Dockyard. *Theor. Appl. Genet.* **64**, 197 (1983). doi:10.1007/BF00303764

34. A. G. Searle, Gene frequencies in London's cats. *J. Genet.* **49**, 214 (1949). doi:10.1007/BF02986074

35. N. B. Todd, Cats and commerce. *Sci. Am.* **237**, 100 (1977). doi:10.1038/scientificamerican1177-100

36. W. E. Johnson *et al.*, The late Miocene radiation of modern Felidae: A genetic assessment. *Science* **311**, 73 (2006). doi:10.1126/science.1122277 Medline