

Supplementary Information for:

The Genetics of Tiger Pelage Color Variations

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Supplementary Discussion

Melanin content in tiger hairs

The melanin content in the zigzag hairs from the background non-striped region of a golden tiger was compared to that of a wild-type orange tiger via spectrophotometric measurement. A significant decrease in eumelanin in the golden tiger's hairs (Figure S1B) was consistent with its observed reduced degree of black pigmentation (Figure 1B). Intriguingly, pheomelanin in the golden tiger's hairs also showed a slight reduction (Figure S1B) despite its prolonged central agouti band, suggesting a likely lower density of pheomelanosomes in the golden tiger's fur, as evident in the overall lighter color of its agouti band (Figures 1A, S1A). It seems surprising yet reasonable that golden tigers with a prolonged pheomelanin band exhibit an overall decrease in pheomelanin content. *ASIP* expression switches pigment synthesis from eumelanin to pheomelanin by inhibiting MC1R signaling [1, 2]. However, with an increased level of *ASIP* expression or the extended presence of *ASIP*, MC1R signaling would be further decreased [2], leading to a reduction in MC1R-associated TYR activity and hence a lower level of pheomelanogenesis. Therefore, the golden tiger blonde color was most likely caused by a lower density of pheomelanosomes as well as a prolonged agouti band in hairs.

Implications for tiger genomic diversity and conservation

In humans, *CORIN* is primarily expressed in cardiac myocytes and regulates blood pressure by converting atrial natriuretic peptide precursor (pro-ANP) into its

mature form (ANP) [3, 4]. Mutations in *CORIN* have been associated with human hypertension and heart failure [5-7], symptoms that have not historically been recorded in golden and snow white tigers, although this could simply be due to a lack of studies. Alternatively, the p.H587Y mutation might not affect the efficiency of *CORIN* in activating natriuretic peptide in tigers, but further studies are needed in this regard. It is also noteworthy that golden and snow white tigers tend to grow larger than their wild-type counterparts in the same captive environment (Dong GX, personal communications). Intriguingly, *CORIN* has been proposed to regulate body weight by degrading agouti-related protein (AGRP) [8]. AGRP is expressed in the hypothalamus and antagonizes melanocortin receptor 4 (MC4R), which regulates feeding and metabolism [9, 10]. Although *CORIN* is not expressed in the brain [3], it may be able to reach the hypothalamus through blood circulation [11]. The potential association between *CORIN* and body mass increases in animals merits further examination.

The *SLC45A2*-associated white tiger morph is a viable, naturally occurring genetic polymorphism that has persisted for centuries in Bengal tigers [12]. Throughout history, white tigers were sighted sporadically across the Indian subcontinent, many of which were captured or shot as mature adults, which provides evidence that they are 'normal' and able to survive and reproduce [13]. The reasons for the extinction of wild white tigers are undoubtedly the same as those that drove the widespread decline of wild tigers in general, rather than the genetic abnormalities. Indeed, although some mutations in *SLC45A2* have been linked to a class of human

oculocutaneous albinism, various natural polymorphisms in *SLC45A2* occur in people, horses, quail and chickens without causing physiological defects other than hypopigmentation [14, 15]. The physiological effects of the *CORIN* variant on golden and snow white tigers remain unclear, considering the protein's critical involvement in multiple cellular and developmental pathways and the fact that confirmed records in the wild are much less common than in the white tiger.

Well-managed captive populations of wild animals can assist in education, research and fundraising, and have been justified as a genetic reservoir for wild animals and insurance against extinction [16]. However, deliberate inbreeding to maintain recessive traits in captivity have led to many undesirable traits associated with captive white, golden and snow white tigers. Most are probably due to improper husbandry, inbreeding depression and general maladies experienced by captive animals [17].

Materials and Methods

Ethical Statement

Biological samples used in the study were recruited in full compliance with the Convention on International Trade in Endangered Species (CITES) and other relevant permissions issued to S. J. Luo at the School of Life Sciences, Peking University, by the State Forestry Administration of China. All biological samples were collected during veterinary examinations for health and physical conditions, and animals were handled following the general animal welfare code of practice.

Biological samples

Fifty-one blood samples from 11 wild-type orange, 9 golden, 18 white and 13 snow white tigers were collected from Chimelong Safari Park, Guangzhou, China. Six blood samples from two white and four orange tigers were collected from Shanghai Zoo, Shanghai, China. Forty-three hair samples from 17 orange, 7 golden, 10 white and 9 snow white tigers were collected during grooming from Tiger Preserve, Myrtle Beach, USA. In addition, 96 DNA samples from unrelated animals, including 90 orange and 6 white tigers, were obtained from the DNA collection of the Laboratory of Genomics Diversity, NCI-Frederick, MD. Information regarding all samples is detailed in Table S1.

To obtain cDNA of the identified gene, one skin biopsy sample from a stillborn kitten was collected from Yongkang Veterinary Clinic, Beijing, and stored at -80°C.

Genomic DNA was extracted from blood or hair samples using the DNeasy Blood and Tissue Kit (QIAGEN) and following the manufacturer's instructions. Total RNA was extracted from the cat skin sample with TRIzol (Invitrogen), and cDNAs were subsequently obtained through RT-PCR with the Superscript® III First-Strand Synthesis System (Invitrogen).

Visual examination of tiger hair morphology

To examine the morphology of the tiger hairs, clusters of full-length hairs were hand plucked from individuals representing all four tiger color morphs at Chimelong

Safari Park, Guangzhou, China. At least four clusters of hairs from the torso flank were taken from the anesthetized animal. Except for the stripeless snow tiger, hairs from both stripes and outside the stripes (background fur) were collected. Individual hairs were isolated from each hair cluster and were observed and photographed using a Leica M125 microscope system with 8x zoom lens under the incident light conditions. The length of each hair section (black tip, sub-apical yellow band and black base) was manually measured on the 8x zoomed screen. In total, 89 and 87 zigzag hairs from the background fur of one orange tiger and one golden tiger, respectively, were measured.

Spectrophotometric determination of eumelanin and pheomelanin

Spectrophotometric assays were conducted to quantitatively measure eumelanin [18] and pheomelanin [19] in different color morphs of tigers. Four specimens of plucked hairs, from stripes and background of a golden tiger and an orange tiger, respectively, were prepared and measured. In addition, plucked hairs from the background fur of a white tiger were used as blank control in both eumelanin and pheomelanin assays. The five hair samples each weighed 7 mg and were homogenized with a glass homogenizer in ddH₂O to a final concentration of 20 mg/mL at 350 μ L and then aliquoted for subsequent assays of eumelanin (absorbance at 350 nm, A_{350}) and pheomelanin (absorbance at 400 nm, A_{400}) with a BioTek Eon spectrophotometer as described previously. The final result of eumelanin or pheomelanin was normalized to the initial hair quantity, subtracted from the white

tiger hair's reading of eumelanin or pheomelanin, and averaged across three replications.

Candidate gene sequencing

Four pigmentation genes (*MC1R*, *ASIP*, *TYR* and *TYRP1*) known to be involved in the coat color genetics of domestic cats were selected as candidate genes for the tiger *WB* locus [20, 21]. Coding regions of the four genes were amplified and sequenced (Table S2) in unrelated individuals, including golden (n=1), snow white (n=1) and orange (n=6) tigers, following PCR and Sanger sequencing procedures as previously described [15]. No variation associated with *WB* was identified.

Gene mapping via genome-wide association study (GWAS)

Nineteen individuals, including golden (n=1), snow white (n=8) and white (n=10) tigers, were assembled for mapping the *wideband* (*WB* locus) gene. Golden and snow white tigers homozygous at the *WB* locus (*wb/wb*, n=9) were carefully selected from Chimelong Safari Park to represent the GWAS case group. The control group was represented by white tigers that were heterozygous at the *WB* locus (*WB/wb*, n=10). These individuals were selected from the same extended family with the *wb/wb* tigers according to zoo breeding records to ensure a similar genetic background with the case group.

Whole-genome sequencing (WGS) was completed in three *wb/wb* tigers (one golden and two snow white) in our previous study [15]. Restriction-site-associated

DNA (RAD) sequencing was applied to the other 16 individuals. Restriction site *Pst*I was selected for RAD sequencing to ensure a 1/20 kb genome-wide SNP density. A genomic paired-end library for RAD-seq was constructed following protocols described previously [15] and sequenced on an Illumina HiSeq 2000 sequencer. Each RAD library was sequenced to an approximate 11-fold depth with an average of 4.39 Gb of data (Table S3). The RAD-seq and WGS were performed at the Biodynamic Optical Imaging Center (BIOPIC) at Peking University.

Procedures for genome sequencing data processing, including adapter trimming, read alignment and SNPs calling, were performed following the workflow described previously [15]. SNPs with data from at least 17 of the 19 individuals were selected for GWAS analysis. The eight snow white tiger and one golden tiger were set as the case group, and the 10 white tigers were set as the control group. GWAS analysis was performed in PLINK 1.07 [22], and the significance of the genotype-phenotype association was calculated by Fisher's exact test under the recessive model. SNPs with a strong association ($p < 0.0001$) were further examined for the genotypes of the 19 tigers. SNPs with a genotype that failed to match the recessive inheritance pattern of the golden trait were considered as false positive signals and discarded. Scaffolds with positive association signals were classified as candidate scaffolds.

Candidate scaffolds were mapped to the cat genome (*Felis_catus* 6.2) [23] to determine conserved syntenies. Haplotypes of the 19 individuals across the candidate scaffolds were inferred using PHASE 2.1 [24] and combined based on the scaffold syntenies. The haplotype block shared by the nine individuals from the case group was

designated as the putative region for the *WB* locus.

Identification of the feline *wideband* gene

Tiger coding exons within the candidate region were obtained from the tiger reference genome annotation and subsequently refined by aligning with the available human, mouse and dog CDS. In addition to the genome data of the three *wb/wb* tigers, the WGS data of 30 orange tigers (unrelated to any golden tiger lineages) from another independent study were also analyzed together. SNPs/indels from non-coding regions were first excluded, and those not fixed in the three *wb/wb* tigers were also excluded. To eliminate common variations within tiger populations, SNPs/indels presented in any of the 31 orange tigers were further excluded. SNPs/indels causing amino acid changes from the remaining SNPs/indels within the candidate region were selected and considered the putative mutation(s).

The potential impact of non-synonymous substitutions in coding regions was evaluated by multivariate analysis of protein polymorphism (MAPP) [25], which considers both evolutionary constraints and the physicochemical properties of amino acid residues; a MAPP score greater than 10 suggests a likely impact of such changes on protein function.

The putative mutation was then validated in an extended sample set of 197 unrelated tigers, including 123 orange, 16 golden, 36 white and 22 snow white tigers. Primers (Table S2) to amplify the candidate *golden* gene were designed based on the tiger reference genome with Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/primer3/>).

PCR and sequencing procedures followed protocols previously described. In addition, the *SLC45A2* p.A477V variant was also examined in the same set of tiger samples.

Functional validation of the *CORIN* p.H587Y mutation

Because sequences of *CORIN* from either cat or tiger were unavailable, we obtained the full cDNA of cat *CORIN* by RT-PCR with a cat skin sample. Sequences of tiger *CORIN* and *ASIP* cDNA were then assembled by aligning cat *CORIN* and cat *ASIP* (NM_001009190) to the tiger reference genome [7]. The cDNAs of tiger *CORIN* and tiger *ASIP* were synthesized accordingly and ligated to the expression vector p3XFLAG-CMV-14, with three tandem FLAG epitopes fused to the C-terminal end. The tiger *CORIN* p.H587Y mutant was obtained by site-directed mutagenesis.

Plasmids containing *CORIN*, the *CORIN* p.H587Y mutation and *ASIP* were transfected into human HEK 293 cells, and *CORIN* and the *CORIN* p.H587Y mutation were co-transfected with *ASIP*. The culture medium was replaced after 18 hours with fresh medium, and the culture supernatants were harvested after another 12 hours. To detect *CORIN*'s effect on the secreted *ASIP*, the medium of *ASIP*-expressing cells was mixed with *CORIN*-variant-expressing cells and cultured for another 3 and 6 hours. HEK 293 cells were lysed in a buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Triton X-100. The expression of tiger *CORIN*, *CORIN* p.H587Y, and *ASIP* from the cell lysates and medium were detected by Western blotting using anti-FLAG antibody.

Supplementary References

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Supplementary Tables and Figures

Table S1. Tiger samples used in the study.

Table S2. Primers for PCR amplification and Sanger sequencing.

Table S3. Summary of RAD-seq and WGS in 19 tigers for GWAS.

Table S4. Genes located within the 9.2 Mb region associated with the WB locus.

Figure S1. **(A)** Photographic and schematic illustration of tiger zigzag hairs. **(B)**

Spectrophotometric measurement of eumelanin and pheomelanin in agouti zigzag hairs from orange and golden tigers. The value was calculated from three replicates using non-pigmented hairs from a white tiger's background fur as the baseline blank.

(C) Association of the "wideband" trait on tiger genome scaffolds 1457 and 97. The scaffold orientation is indicated by arrows, and the *wideband*-linked genomic region is specified.

Table S1. Tiger samples used in the study.

Animal	Color	CORIN SLC45A2		Owner & Contact	Birth Status	Sex	Note
		(C>T)	(C>T)				
GZXJ003	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ005	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>white</i>
GZXJ026	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ027	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ028	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ029	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ030	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>white</i>
GZXJ033	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ039	Golden	TT	CT	Chimelong Safari Park, China, Dong GX	captive	F	WGS for <i>wideband & white</i>
Brahman	Golden	TT	CC	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Krupa	Golden	TT	CT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Mata	Golden	TT	CT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Muktari	Golden	TT	CT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Padha	Golden	TT	CT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Raga	Golden	TT	CC	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Rama	Golden	TT	CC	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
GZXJ004	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ006	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ031	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ032	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ034	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	M	WGS for <i>wideband & white</i>
GZXJ035	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	M	WGS for <i>wideband & white</i>
GZXJ036	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ037	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>wideband</i>
GZXJ038	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>white</i>
GZXJ046	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ047	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ048	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>wideband</i>
GZXJ050	Snow	TT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
Amar	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Joyti	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Kylash	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Raksha	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Saraswati	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Shankar	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Simbad	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Sri	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Sundri	Snow	TT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
GZXJ001	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	M	individual for validation
GZXJ002	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	F	individual for validation
GZXJ007	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ008	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ009	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	M	individual for validation
GZXJ010	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	M	individual for validation
GZXJ011	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	M	individual for validation
GZXJ012	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	F	individual for validation
GZXJ013	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	F	individual for validation
GZXJ014	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	F	individual for validation
GZXJ040	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>wideband</i>
GZXJ041	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ042	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>wideband</i>
GZXJ043	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ044	White	CT	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>wideband</i>
GZXJ045	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
GZXJ049	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	F	RAD-seq for <i>wideband</i>
GZXJ051	White	CC	TT	Chimelong Safari Park, China, Dong GX	captive	M	RAD-seq for <i>wideband</i>
Bhavantu	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Bindi	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Ganga	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Ishwari	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	?	individual for validation
Lolita	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Marayani	White	CC	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	M	individual for validation
Samasta	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Shakra	White	CC	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Shakti	White	CT	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
Uma	White	CC	TT	Tiger Preserve Myrtle Beach, USA, Antle B	captive	F	individual for validation
PTIP166	White	CC	TT	Peking University, China, Luo SJ	captive	M	individual for validation
PTIP167	White	CC	TT	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP17	White	CC	TT	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP223	White	CC	TT	Peking University, China, Luo SJ	captive	M	individual for validation
PTIP289	White	CT	TT	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP90	White	CC	TT	Peking University, China, Luo SJ	captive	F	individual for validation
SHZO05	White	CT	TT	Shanghai Zoo, China, Zheng JQ	captive	M	individual for validation

Animal	Color	CORIN SLC45A2		Owner & Contact	Birth		
		(C>T)	(C>T)		Status	Sex	Note
PTIP263	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP264	Orange	CC	CC	Peking University, China, Luo SJ	wild	F	individual for validation
PTIP269	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP271	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP272	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP273	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP274	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP276	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP279	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP282	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP283	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP285	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP286	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP288	Orange	CC	CC	Peking University, China, Luo SJ	captive	F	individual for validation
PTIP293	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP296	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP300	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP301	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP302	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP303	Orange	CC	CC	Peking University, China, Luo SJ	captive	M	individual for validation
PTIP305	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP306	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP307	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP309	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP310	Orange	CC	CC	Peking University, China, Luo SJ	wild	F	individual for validation
PTIP311	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP312	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP313	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP315	Orange	CC	CC	Peking University, China, Luo SJ	wild	M	individual for validation
PTIP316	Orange	CC	CC	Peking University, China, Luo SJ	unk	unk	individual for validation
PTIP320	Orange	CC	CC	Peking University, China, Luo SJ	captive	M	individual for validation
PTIP327	Orange	CC	CC	Peking University, China, Luo SJ	unk	unk	individual for validation
PTIP330	Orange	CC	CC	Peking University, China, Luo SJ	unk	unk	individual for validation
PTIP331	Orange	CC	CC	Peking University, China, Luo SJ	unk	unk	individual for validation
PTIP332	Orange	CC	CC	Peking University, China, Luo SJ	unk	unk	individual for validation
PTIP36	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP38	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP65	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP70	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP72	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP83	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP84	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
PTIP88	Orange	CC	CC	Peking University, China, Luo SJ	unk	M	individual for validation
PTIP99	Orange	CC	CC	Peking University, China, Luo SJ	unk	F	individual for validation
SHZO01	Orange	CC	CC	Shanghai Zoo, China, Zheng JQ	captive	M	individual for validation
SHZO02	Orange	CC	CC	Shanghai Zoo, China, Zheng JQ	captive	M	individual for validation
SHZO03	Orange	CC	CC	Shanghai Zoo, China, Zheng JQ	captive	M	individual for validation
SHZO04	Orange	CC	CC	Shanghai Zoo, China, Zheng JQ	captive	M	individual for validation

1. unk = unknown

Table S2. Primers for PCR amplification and Sanger sequencing.

Gene and Amplicon	Amplicon size (bp)	Forward primer (5' to 3')	Reverse primer (5' to 3')	Reference
<i>MC1R</i> -ex-a	429	CATTGTGCCTGAGCTGACAT	GTCTCCAGCACACTGCTCAC	Xu <i>et al.</i> (2013)
<i>MC1R</i> -ex-b	399	CTGCACTCGCCCATGTATTA	CACCAGCATGGCTACAAAGA	Xu <i>et al.</i> (2013)
<i>MC1R</i> -ex-c	472	TCGCCTACTACGATCACACG	GCCATAGGATATCCCCACCT	Xu <i>et al.</i> (2013)
<i>ASIP</i> -ex1	418	TCATCAGTTCCCTTTTCCTG	GGGAGCACGTTTGACATCTT	Xu <i>et al.</i> (2013)
<i>ASIP</i> -ex2	348	CTCTTCTCCACACCCTGAG	CCCTTAGCTCTCTGGGCTTC	Xu <i>et al.</i> (2013)
<i>ASIP</i> -ex3	450	AGAGTTCCCAAGGCTGAGGT	AAATGCAAGAAAGCCACACC	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex1-a	366	GCAGGAGAGATGCAGAGGAG	TTGGAGGGTCTGAAATCTGG	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex1-b	386	TGTTCCACAGCAACAAGAA	TTTGGCAACTTCATGGGATT	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex1-c	371	TCGCTTCTCTGTGCAGTTTG	GATCCGTGAAGACGAGGGTA	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex2	310	CATGATTTCAGAAAAACAGAAGAAA	TCTCTAGCCACAACCTCTTAACA	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex3	356	TTTGCCTCCAACCTCCTTACG	TTTCAACTACCAGGGCCAAC	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex4	298	TTGGCATCCTTCCATGTCTT	GGCTTTGGGGATAGCATTTGT	Xu <i>et al.</i> (2013)
<i>TYR</i> -ex5	423	TAGCAGAGCTGGCATTCAAA	AAAATGAGGTCAACCTTGTTGG	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex1	399	GGGAAGGGAATCATGTGCTA	CATTCGTTAGCAGGTCATGG	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex2	511	TGCCATTTTGTCTTCTCCTT	TGTACATGCAGATCCCCACT	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex3	399	GTGGCACATTTTCACGTTTG	CTGTCGGGGACCTGAAAAG	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex4	355	CAGGGAGAAAACCAAGCAAA	TGAGGACTTTCAGACAGCACA	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex5	249	TTCTGTAAGTGTTCCTATAACCTGT	TGTGACAACCTAGATAAAATCAAAGCA	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex6	289	AGACATTGGCAACGGTTTTTC	CCATACCACCTGAACGGTCT	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex7	217	TTAACAAGATGTCTTTGGCATATTT	TTTTGGTAATTTCCACAGAAAAGA	Xu <i>et al.</i> (2013)
<i>TYRP1</i> -ex8	250	TGTTCTCCTTTTGTTTTTTCACAG	CAGAGAATTGGTCGTTTGTCA	Xu <i>et al.</i> (2013)
<i>SLC45A2</i> -ex7	288	TGTTCCCTGTGTTCCCTTGC	CCCTTCACTGTGTTGGAGGT	Xu <i>et al.</i> (2013)
<i>Corin</i> -ex13	214	TGCTTCCAGGTTTCTGTCC	TCCTAGCCAGGCATTAACGTA	this study
<i>Corin</i> -cds	3311	GAGGAGCAAGTCGTCCGTAG	GGAAGCACTCCACAGTCAA	this study

Table S3. Summary of RAD-seq and WGS in 19 tigers for GWAS.

Sample ID	Raw bases (Gb)	Unique mapped (Gb)	Unique mapped ratio	WGS Seq depth (unique maps)	Unique mapped read pairs (M)	No. <i>PstI</i> sites ¹	<i>PstI</i> coverage ratio	<i>PstI</i> site coverage depth ²	SNP count ³	SNP ratio
RAD-seq										
GZXJ036	7.270	4.142	56.97%	n/a	21.395	671,459	90.80%	14.466	626,233	70.64%
GZXJ037	4.495	2.634	58.60%	n/a	13.597	656,863	88.82%	9.193	468,226	52.81%
GZXJ046	4.634	3.579	77.23%	n/a	18.462	689,209	93.20%	12.482	645,676	72.83%
GZXJ047	5.255	4.065	77.36%	n/a	20.977	685,594	92.71%	14.183	667,337	75.27%
GZXJ048	4.753	3.705	77.95%	n/a	19.113	684,725	92.59%	12.923	631,050	71.18%
GZXJ050	3.537	2.739	77.43%	n/a	14.185	665,320	89.97%	9.591	530,173	59.80%
GZXJ007	3.992	3.078	77.08%	n/a	15.916	686,072	92.77%	10.761	595,826	67.21%
GZXJ008	3.864	2.986	77.28%	n/a	15.428	657,450	88.90%	10.431	581,613	65.60%
GZXJ040	3.866	2.980	77.07%	n/a	15.408	660,228	89.28%	10.418	559,905	63.15%
GZXJ041	3.844	2.992	77.84%	n/a	15.466	678,701	91.78%	10.457	580,632	65.49%
GZXJ042	4.270	3.315	77.62%	n/a	17.108	675,690	91.37%	11.567	610,789	68.89%
GZXJ043	4.833	3.739	77.36%	n/a	19.285	690,705	93.40%	13.039	649,919	73.31%
GZXJ044	4.613	3.553	77.02%	n/a	18.331	670,745	90.70%	12.394	650,634	73.39%
GZXJ045	4.315	3.355	77.76%	n/a	17.298	665,133	89.94%	11.696	631,453	71.22%
GZXJ049	4.659	3.619	77.67%	n/a	18.666	685,771	92.73%	12.621	643,323	72.56%
GZXJ051	2.038	1.624	79.68%	n/a	8.398	617,060	83.44%	5.678	356,051	40.16%
Average	4.390	3.256	75.12%	n/a	16.815	671,295	90.77%	11.369	589,303	66.47%
Total	70.239	52.103	74.18%	n/a	269.034	739,518	100%	181.898	886,564	100%
WGS										
GZXJ034	85.036	74.027	87.05%	30.558						
GZXJ035	78.945	70.798	89.68%	29.608						
GZXJ039	82.319	74.385	90.36%	31.108						
Average	82.100	73.07	89.03%	30.558						

1. A *PstI* site was counted only when both upstream and downstream of the site were covered by the P1 reads of RADSeq data.
2. *PstI* site coverage depth was calculated by uniquely mapped read pairs divided by number of *PstI* sites.
3. A SNP was counted only when data was available from 6 samples and exhibited polymorphism among the samples.

Table S4. Genes located within the 9.2 Mb region associated with the *WB* locus

Scaffold	start	end	Gene	Description
scaffold1457	1702057	1714742	<i>OCIAD2</i>	OCIA domain containing 2.
scaffold1457	1723169	1748562	<i>OCIAD1</i>	OCIA domain containing 1.
scaffold1457	1758261	1758548	<i>RPS21</i>	Ribosomal protein S21.
scaffold1457	1907736	2054288	<i>FRYL</i>	FRY-like.
scaffold1457	2059201	2061054	<i>ZARI</i>	Zygote arrest 1, the maternal effect gene is oocyte-specific and encodes a protein that is thought to function in the initiation of embryogenesis.
scaffold1457	2064914	2069943	<i>SLC10A4</i>	Solute carrier family 10, member 4.
scaffold1457	2136797	2174285	<i>SLAIN2</i>	SLAIN motif family, member 2
scaffold1457	2303541	2391690	<i>TEC</i>	The protein encoded by this gene belongs to the Tec family of non-receptor protein-tyrosine kinases containing a pleckstrin homology domain.
scaffold1457	2413405	2455308	<i>TXK</i>	TXK tyrosine kinase.
scaffold1457	2474274	2499970	<i>NIPAL1</i>	NIPA-like domain containing 1
scaffold1457	2531986	2552038	<i>CNGA1</i>	The protein encoded by this gene is involved in phototransduction.
scaffold1457	2567931	2635094	<i>NFXL1</i>	Nuclear transcription factor, X-box binding-like 1.
scaffold1457	2643215	2892269	<i>CORIN</i>	CORIN, a serine protease, regulates blood pressure by converting pro-ANP to active ANP, and modifies pigmentation through agouti pathway.
scaffold1457	2896348	2972767	<i>ATP10D</i>	ATPase, Class V, Type 10D.
scaffold1457	3024887	3032276	<i>COMMD8</i>	COMM domain containing 8.
scaffold1457	3053059	3152623	<i>GABRB1</i>	GABRB1 is a member of the GABA-A receptor gene family of heteromeric pentameric ligand-gated ion channels.
scaffold1457	3457791	3522485	<i>GABRA4</i>	GABRA4 is a member of the GABA-A receptor gene family of heteromeric pentameric ligand-gated ion channels
scaffold1457	3695631	3695858	<i>COX7B2</i>	Cytochrome c oxidase subunit VIIb2
scaffold1457	3718243	3719410	<i>AMD1</i>	This gene encodes an important intermediate enzyme (adenosylmethionine decarboxylase 1) in polyamine biosynthesis.
scaffold1457	3957480	4080396	<i>GABRA2</i>	GABRA2 is a member of the GABA-A receptor gene family of heteromeric pentameric ligand-gated ion channels.
scaffold1457	4180616	4245668	<i>GABRG1</i>	GABRG1 is a member of the GABA-A receptor gene family of heteromeric pentameric ligand-gated ion channels.
scaffold1457	5191460	5191870	<i>AHCY</i>	This gene encodes adenosylhomocysteinase, which regulates the intracellular S-adenosylhomocysteine (SAH) concentration thought to be important for transmethylation reactions.
scaffold1457	5247021	5247604	<i>PFN1</i>	This gene encodes a member of the profilin family of small actin-binding proteins. The encoded protein plays an important role in actin dynamics by regulating actin polymerization in response to extracellular signals.
scaffold1457	5313895	5340309	<i>GNPDA2</i>	Glucosamine-6-phosphate deaminase 2 is an allosteric enzyme that catalyzes the reversible reaction converting D-glucosamine-6-phosphate into D-fructose-6-phosphate and ammonium.
scaffold1457	5344182	5365639	<i>GUF1</i>	GUF1 GTPase homolog.
scaffold1457	5387429	5411941	<i>YIPF7</i>	Yip1 domain family, member 7.
scaffold1457	5499902	5500279	<i>CALM1</i>	This gene encodes a member of the EF-hand calcium-binding protein family.
scaffold1457	5601425	5852257	<i>KCTD8</i>	Potassium channel tetramerization domain containing 8
scaffold97	5775335	5901979	<i>GRXCR1</i>	This gene encodes a protein which contains GRX-like domains; these domains play a role in the S-glutathionylation of proteins and may be involved in actin organization in hair cells.
scaffold97	5660849	5661106	<i>MRPL42</i>	Mitochondrial ribosomal protein L42.
scaffold97	5367350	5559987	<i>ATP8A1</i>	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1.
scaffold97	5355011	5355451	<i>SHISA3</i>	Shisa family member 3.
scaffold97	5195908	5198111	<i>TOM1L1</i>	TOM1L1 belongs to a family of TOM1 (604700)-related proteins involved in endocytosis.
scaffold97	5127140	5151148	<i>BEND4</i>	BEN domain containing 4.
scaffold97	5027882	5083207	<i>SLC30A9</i>	Solute carrier family 30 (zinc transporter), member 9.
scaffold97	4965341	4984434	<i>TMEM33</i>	Transmembrane protein 33.
scaffold97	4799268	4801620	<i>PHOX2B</i>	Paired-like homeobox 2b functions as a transcription factor involved in the development of several major noradrenergic neuron populations and the determination of neurotransmitter phenotype.
scaffold97	4543535	4743582	<i>LIMCH1</i>	LIM and calponin homology domains 1.
scaffold97	4340395	4353221	<i>UCHL1</i>	The protein encoded by this gene belongs to the peptidase C12 family. This enzyme is a thiol protease that hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin.
scaffold97	3921344	4110223	<i>APBB2</i>	The protein encoded by this gene interacts with the cytoplasmic domains of amyloid beta (A4) precursor protein and amyloid beta (A4) precursor-like protein 2.
scaffold97	3612440	3622668	<i>RBM47</i>	RNA binding motif protein 47.
scaffold97	3543968	3556619	<i>CHRNA9</i>	This gene is a member of the ligand-gated ionic channel family and nicotinic acetylcholine receptor gene superfamily. It encodes a plasma membrane protein that forms homo- or hetero-oligomeric divalent cation channels.
scaffold97	3471842	3472404	<i>RHOH</i>	The protein encoded by this gene is a member of the Ras superfamily of guanosine triphosphate (GTP)-metabolizing enzymes.
scaffold97	3342991	3395241	<i>N4BP2</i>	NEDD4 binding protein 2 can bind to both B-cell leukemia/lymphoma 3 (BCL-3) and neural precursor cell expressed, developmentally downregulated 4, (Nedd4) proteins.
scaffold97	3109775	3237547	<i>PDS5A</i>	The protein encoded by this gene binds to the cohesin complex and associates with chromatin through most of the cell cycle.
scaffold97	2993833	3062282	<i>UBE2K</i>	The protein encoded by this gene belongs to the ubiquitin-conjugating enzyme family. This protein interacts with RING finger proteins, and it can ubiquitinate huntingtin, the gene product for Huntington's disease.
scaffold97	2853674	2894549	<i>SMIM14</i>	Small integral membrane protein 14.
scaffold97	2808961	2825017	<i>UGDH</i>	The protein encoded by this gene converts UDP-glucose to UDP-glucuronate and thereby participates in the biosynthesis of glycosaminoglycans such as hyaluronan, chondroitin sulfate, and heparan sulfate.
scaffold97	2785239	2800594	<i>LIAS</i>	Lipoic acid synthetase, involves in the cell growth.
scaffold97	2780714	2784403	<i>RPL9</i>	Ribosomal protein L9.
scaffold97	2741158	2774128	<i>KLB</i>	Klotho beta is an essential cofactor for FGF21 activity and suggested that the Klotho gene family may have evolved to confer tissue-specific bioactivity on FGF19 (603891) subfamily members.
scaffold97	2643471	2706239	<i>RFC1</i>	This gene encodes the large subunit of replication factor C, a five subunit DNA polymerase accessory protein, which is a DNA-dependent ATPase required for eukaryotic DNA replication and repair.
scaffold97	2534048	2624602	<i>WDR19</i>	This gene encodes a member of the WD repeat protein family. Members of this family are involved in a variety of cellular processes, including cell cycle progression, signal transduction, apoptosis, and gene regulation.
scaffold97	2434396	2499017	<i>KLHL5</i>	Kelch-like family member 5.
scaffold97	2346827	2380860	<i>TMEM156</i>	Transmembrane protein 156.
scaffold97	2255513	2310967	<i>FAM114A1</i>	Family with sequence similarity 114, member A1.
scaffold97	2217896	2220283	<i>TLR6</i>	Toll-like receptor 6 is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity.
scaffold97	2190563	2191732	<i>TLR1</i>	Toll-like receptor 1 is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity.
scaffold97	2163113	2165542	<i>TLR10</i>	Toll-like receptor 10 is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity.
scaffold97	2081886	2098226	<i>KLF3</i>	Kruppel-like factor 3 (basic).
scaffold97	1728297	1728665	<i>RPL41</i>	Ribosomal protein L41.
scaffold97	1429354	1643878	<i>TBC1D1</i>	TBC1D1 is the founding member of a family of proteins sharing a 180- to 200-amino acid TBC domain presumed to have a role in regulating cell growth and differentiation.

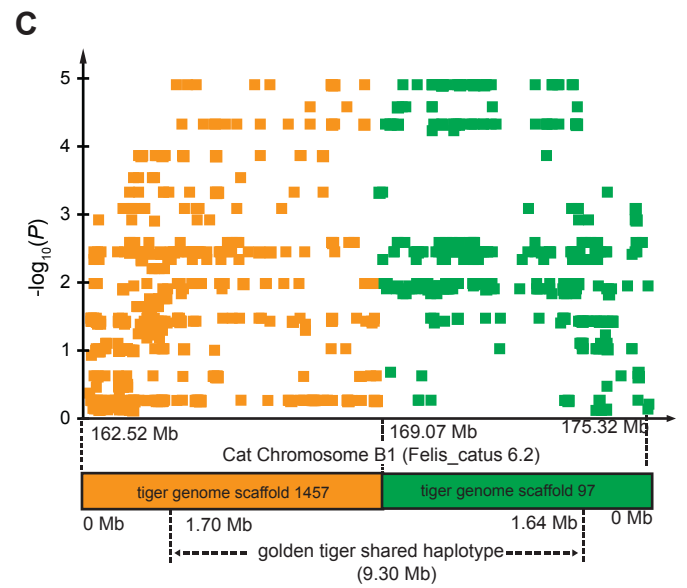
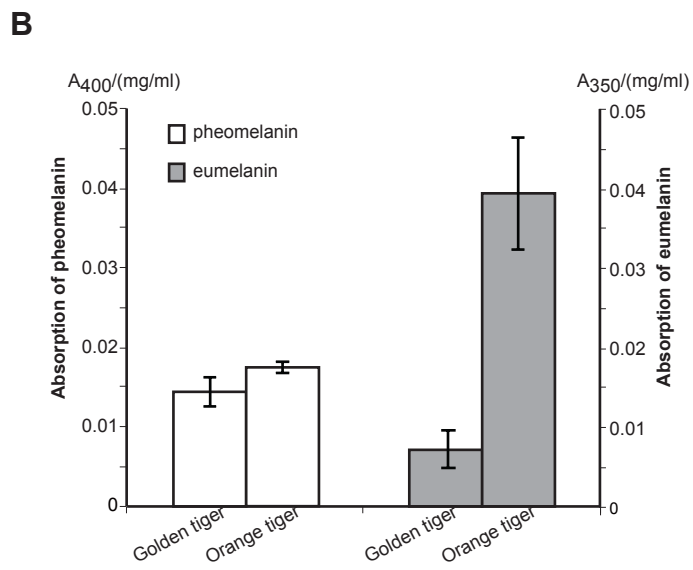
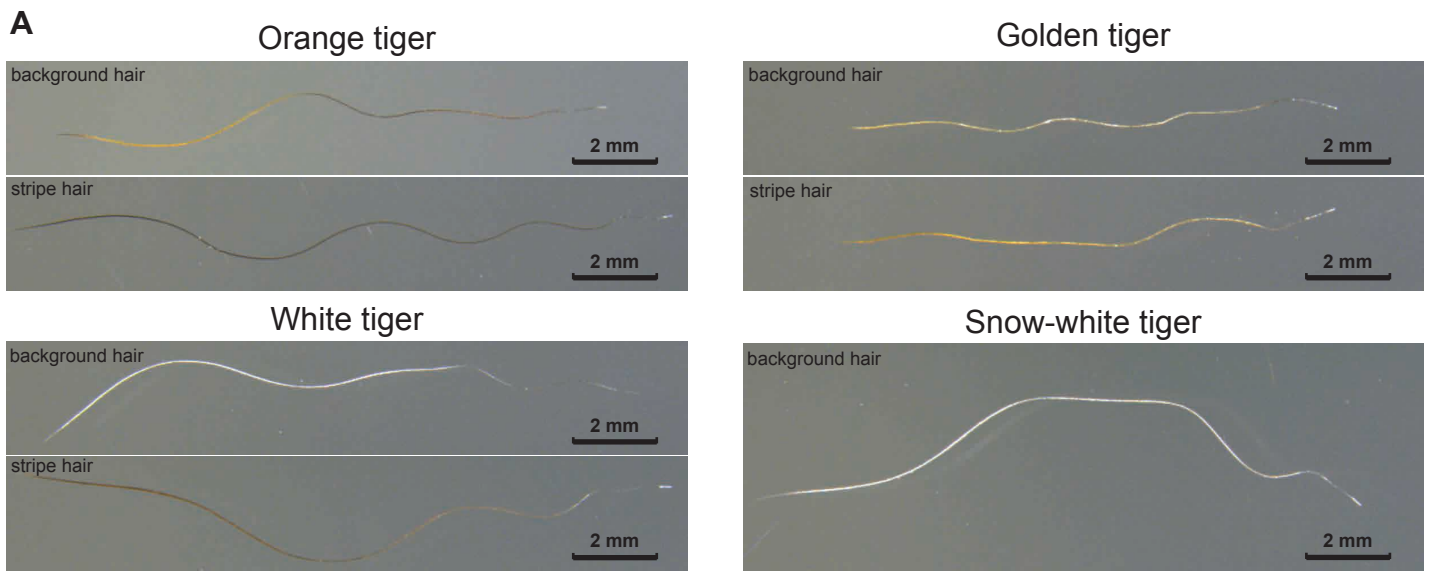


Figure S1