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## 2 **Genetics of white color and iridophoroma in “Lemon Frost” leopard geckos**

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20

### 21 **Abstract**

22 Coloration patterns promote survival and reproductive success in the animal kingdom. Despite  
23 their importance, wide gaps exist in our understanding of the genetic and evolutionary  
24 mechanisms that underpin them. The leopard gecko<sup>1</sup>, *Eublepharis macularius*, is a popular  
25 companion animal, and displays a variety of coloration patterns. We investigated a spontaneous  
26 semi-dominant mutation, known as “Lemon Frost”, that causes extensive white color in leopard

27 gecko skin. Although “Lemon Frost” individuals are aesthetically appealing, more than 80% of  
28 them develop tumors of white color (*i.e.*, iridophoroma) 0.5 to 5 years after birth. To identify the  
29 gene that regulates white color and is likely also responsible for the iridophoroma, we  
30 genotyped 220 animals, including 33 homozygous mutants, with short-read sequencing. We  
31 used synteny, linkage analysis and homozygosity mapping to localize the mutation to a strong  
32 candidate gene, SPINT1<sup>2,3</sup>, a tumor suppressor previously implicated in human skin cutaneous  
33 melanoma (SKCM) as well as in over-proliferation of epithelial cells in mice and zebrafish<sup>4-16</sup>.  
34 Our work establishes the leopard gecko as a tractable genetic system and suggests that a  
35 tumor suppressor in melanocytes in humans can also suppress tumor development in  
36 iridophores in lizards.

37

## 38 **Introduction**

39 Color-producing cells<sup>17-21</sup> contribute to animal coloration and patterns. Some cells, such as  
40 melanocytes, produce pigments chemically. Others, such as iridophores, produce colors  
41 structurally by making crystal platelets<sup>22-25</sup>. Iridophores are not present in mammals, but are  
42 widespread in insects, fish, birds, amphibians and reptiles. Different types of iridophores can  
43 lead to different colors, including blue<sup>26,27</sup> and white<sup>28</sup>. There have been few molecular genetic  
44 analyses of the regulation of chromatophores in cells other than melanocytes. A recent study  
45 found that endothelin signaling regulates iridophore development and proliferation in zebrafish<sup>29</sup>.  
46 In mammals, this pathway is required for melanocyte development<sup>30</sup>, suggesting that signaling  
47 pathways conserved in evolution can be adapted to regulate different types of chromatophores.

48

49 Many reptile species (*e.g.*, geckos, chameleons, snakes) are bred in captivity as companion  
50 animals, and breeders have established morphs with unique colors and patterns<sup>18</sup>. The  
51 inheritance of different color morphs is usually carefully documented by breeders. The common  
52 leopard gecko, *Eublepharis macularius*, is an especially attractive model to study the molecular

53 regulation of coloration because dozens of color and pattern morphs have been established  
54 over the past 30 years of selective breeding. These morphs either intensify a particular color  
55 (Supplementary Figure1 A-I) or rearrange coloration patterns (Supplementary Figure1 J-L). A  
56 draft leopard gecko genome assembly has been published, containing 2.02 Gb of sequence in  
57 22,548 scaffolds, with 24,755 annotated protein-coding genes<sup>1</sup>. Embryonic development *in ovo*  
58 and blastema-based tail regeneration have also been staged and documented in great detail<sup>31-33</sup>.  
59 Here, we took advantage of these established resources and used quantitative genetics to gain  
60 insight into the molecular regulation of white color in leopard geckos.

61

## 62 **Results**

63

### 64 **The Lemon Frost allele is a spontaneous semidominant mutation**

65 A spontaneous mutation occurred in a female hatchling from a cross between two wildtype  
66 leopard geckos. This mutation increased the white color of the leopard gecko, resulting in  
67 brightened white and yellow colors. This unique color morph was named Lemon Frost<sup>34</sup> (Figure  
68 1). A male leopard gecko carrying the *lemon frost* (*lf*) allele, Mr. Frosty (Figure 1B), was crossed  
69 to 12 female leopard geckos of different genetic backgrounds. The F1 progeny, which were  
70 heterozygous for the *lf* allele, were backcrossed to the same maternal lines or intercrossed to  
71 establish a colony of more than 900 animals (Supplementary Figure 2). Homozygous F2  
72 intercross progeny were named super Lemon Frost (Figure 1C). These homozygous mutants  
73 have an accentuated color phenotype and thickened skin, which is most apparent in their  
74 eyelids (Figure 1C, red arrow). Heterozygous Lemon Frost animals were also crossed to  
75 another mutant, Blizzard, which is light yellow without other colors or patterns (Figure 1D). The  
76 homozygous Blizzard progeny carrying the *lf* allele displayed excessive white color in their  
77 heads and trunks, which brightened Blizzard's yellow color (Figure 1E). The *lf* allele also  
78 increased white color in the retina (Figure 1E). The segregation pattern of Lemon Frost in

79 pedigrees is consistent with single-locus Mendelian inheritance (Figure 1F-H). The *If* allele is  
80 semidominant, as homozygous mutants have more pronounced phenotypes than do  
81 heterozygotes (Figure 1B-C and F-H).

82

83 **The *lemon frost* allele leads to iridophoroma, with potential metastasis in homozygous**  
84 **animals**

85 Heterozygous Lemon Frost mutants were recently reported to develop iridophoroma<sup>34</sup>, a tumor  
86 of iridophores. Histopathological examination of the skin samples from homozygous mutants,  
87 with accentuated phenotypes, showed large solid sheaths of round to polygonal neoplastic cells  
88 that efface and expand the normal tissue architecture (Supplementary Figure 3). The cells have  
89 abundant cytoplasm with bright brownish intracytoplasmic pigment. The nuclei are eccentric and  
90 vary from round to fusiform. The white tumor masses stain dark with Hematoxylin and Eosin  
91 (H&E), and remain brightly reflective under dark-field illumination (Supplementary Figure 4A,B),  
92 consistent with their nature as iridophores<sup>26,35-38</sup>. Imaging with Transmission Electron  
93 Microscopy (TEM) showed that the *If* allele led to both increased numbers of neoplastic  
94 iridophores and increased production of reflective platelets within each iridophore<sup>39</sup>  
95 (Supplementary Figure 4C). In addition to skin, other affected organs in homozygous mutants  
96 include liver, eye, and muscle. The interpretation of the widespread neoplastic nodules is that  
97 the tumors are malignant iridophoroma.

98

99 More than 80% of both male and female animals carrying the *If* allele developed white tumors 6  
100 months to 5 years after birth. The tumors manifest as patches of white cells in the skin, which  
101 are most evident on the ventral side of the animal (Figure 2A). The tumor skin can be severely  
102 thickened and leathery (Figure 2B, Supplementary Figure 3). It is resistant to liquid nitrogen  
103 freezing, or to Dounce homogenization, making RNA extraction infeasible. In severe cases in

104 heterozygous mutants, the tumors develop into skin protrusions (Figure 2C, left), which contain  
105 dense white masses (Figure 2C, right). Tumors cover a greater fraction of the skin of  
106 homozygous mutants. Surprisingly, these tumors rarely develop into skin protrusions as in  
107 heterozygous animals. Instead, they manifest as well-demarcated, white, thickened patches on  
108 the ventral skin (Figure 2A), thickened layers of white masses all over the dorsal skin (Figure  
109 2B), white, multifocal, variably sized, well-demarcated nodules in the liver, and patches of white  
110 cells in the oral cavity (Figure 2D).

111

### 112 **Linkage and association analysis in a breeding pedigree**

113 To identify the genetic locus that regulates white color and tumor growth in *Lemon Frost*  
114 mutants, we used restriction site-associated DNA sequencing (RAD-Seq) to genotype 188  
115 animals from the breeding pedigree (Figure 3, Supplementary Figure 2), including 33 super  
116 Lemon Frost (*lfl/fl*), 116 Lemon Frost (*lfl/+*), and 39 wild-type (*+/+*) individuals. We identified a  
117 total of 14,857 variants covering 2,595 scaffolds of the genome assembly. To map the Lemon  
118 Frost locus, we tested the effect of allelic dosage at each marker on white coloration of the  
119 geckos in a standard semi-dominant association mapping framework, accounting for population  
120 structure through the use of marker-based relatedness. We used a p-value threshold of  $7.09e-5$   
121 (Methods) to control the false positive rate at 1%. Forty-eight markers on 31 scaffolds were  
122 significantly associated with white coloration (Supplemental Table). The top two association  
123 signals corresponded to scaffolds 6052 and 996.

124

### 125 **Synteny analysis and homozygosity mapping**

126 Because the gecko genome assembly is highly fragmented, we used synteny to examine  
127 whether the 31 scaffolds associated with coloration belong to a single genomic interval. We  
128 compared the gecko scaffolds to homologous regions of the most closely related species with  
129 chromosome-scale genome assemblies: chicken<sup>40</sup> and human<sup>41</sup>. We found that 17 out of 22

130 scaffolds that have synteny information (including scaffolds 6052 and 996) correspond to one  
131 region on chicken chromosome 5 and human chromosome 15 (Figure 3A-C, Supplemental  
132 Table). The 28 markers on these 17 scaffolds are in linkage disequilibrium (Figure 3B), which  
133 decays with distance when markers are ordered by synteny (Figure 3B). These results indicate  
134 that a single genomic region is associated with the Lemon Frost phenotype, as expected for a  
135 new mutation with a Mendelian segregation pattern.

136

137 To narrow down the location of the causal gene within this genomic region, we used whole  
138 genome sequencing and homozygosity mapping. We pooled DNA from 25 super Lemon Frost  
139 genomes (*lfl/lfl*), 63 Lemon Frost genomes (*lfl/+*), and 71 wildtype geckos (*+/+*) and sequenced  
140 each pool to 30x coverage. We reasoned that the *lfl* mutation in Mr. Frosty and its flanking  
141 variants should form a haplotype that would be found in the super Lemon Frost pool with 100%  
142 frequency, in the Lemon Frost pool with 50% frequency, and would not be seen in the wildtype  
143 pool. We scanned the genome in 10 kb windows and measured the fraction of heterozygous  
144 variants from Mr. Frosty that followed this expected pattern in the pools. This statistic was  
145 highest for a window on scaffold 996 (Supplementary Table, Methods), the main candidate  
146 scaffold from statistical mapping. The expected frequency pattern was observed for 20 of 22  
147 variants in this window (630-640kb on scaffold 996). Four of the top six intervals fall in the  
148 region from 570kb to 640kb on scaffold 996, with the signal decaying with distance away from  
149 this region (Figure 3D,E). The linkage between this region and Lemon Frost was replicated in an  
150 independent 3-generation backcross between Mr. Frosty and a Sunburst Tangerine morph  
151 (Figure 4). These results indicate that scaffold 996 contains the Lemon Frost mutation.

152

### 153 **SPINT1 is a strong candidate gene for the Lemon Frost phenotype**

154 The genomic interval spanning positions 570kb-640kb on scaffold 996 contains a single gene,  
155 SPINT1. SPINT1 (serine peptidase inhibitor, Kunitz type 1), also known as hepatocyte growth

156 factor activator inhibitor type 1 (HAI-1), is a transmembrane serine protease inhibitor expressed  
157 mainly in epithelial cells<sup>2,3,16</sup>. It is the only gene in the larger associated region reported to be a  
158 suppressor of epithelial cell tumors in model organisms and in humans<sup>2,4-14,42</sup>. Because the  
159 breeding and transmission data indicate that the *lf* allele arose from a single spontaneous  
160 mutation, we reasoned that a mutation disrupting SPINT1 causes the over-proliferation of white-  
161 colored skin cells in Lemon Frost geckos.

162

163 The Lemon Frost SPINT1 allele differs from the reference genome assembly at two positions in  
164 the exons, as well as at 147 positions in the introns and the 3'UTR (Supplemental Table). This  
165 large number of variants is a consequence of differences in genetic background between Mr.  
166 Frosty's parents and the non-Lemon Frost individual used to generate the reference, and makes  
167 it challenging to identify the causal mutation. Both differences in the coding sequence of  
168 SPINT1 are synonymous. Notable differences in non-coding regions include 7 large  
169 insertion/deletions (indels) in the introns and a 13-nucleotide insertion in the 3'UTR  
170 (CAAGTGTATGTAT). Indels in introns and promoters of SPINT1 have been reported to lead to  
171 loss of SPINT1 function in fish and mice<sup>8,9,6</sup>.

172

173 Sequencing of RNA extracted from normal gecko skin and from skin peripheral to tumors in  
174 homozygous mutants confirmed that SPINT1 is expressed in this tissue (Supplemental Figure  
175 5). However, we did not observe a significant difference between homozygous mutants and  
176 wildtype geckos in SPINT1 mRNA levels or splicing patterns. This result suggests that the  
177 putative causal mutation in SPINT1 may alter translation or protein activity, rather than  
178 transcription. Alternatively, the mutation might reduce SPINT1 expression only in tumors, which  
179 are refractory to RNA extraction as noted above.

180

181 **Discussion**

182 Several lines of evidence support our hypothesis that a defect in SPINT1 causes iridophoroma  
183 in Lemon Frost geckos. First, SPINT1 function is dosage-dependent, consistent with our  
184 observation that Lemon Frost is a semi-dominant phenotype. In humans, carcinoma tissues *in*  
185 *vivo* and carcinoma-derived cell lines *in vitro* have reduced SPINT1 on the cell membrane<sup>15,43</sup>  
186 through enhanced shedding of the extracellular domain or decreased mRNA or protein  
187 expression. Reduced expression of SPINT1 has been associated with a negative prognosis of  
188 human Skin Cutaneous Melanoma (SKCM)<sup>4</sup> and pancreatic ductal adenocarcinoma<sup>13</sup>.  
189 Knockdown of SPINT1 expression by siRNA in cancer cell lines led to increased invasion or  
190 metastasis<sup>14,15,44</sup>. Second, loss of SPINT1 function in fish and mice leads to tumor formation in  
191 epithelial cells. In mice, homozygous deletion of SPINT1 leads to disrupted placental basement  
192 membranes and embryonic lethality<sup>9,11</sup>. Rescued mosaic animals developed scaly skin with  
193 hyperkeratinization<sup>12</sup>. Intestine-specific deletion of SPINT1 leads to increased tumor growth of  
194 intestine epithelium<sup>10</sup>. Increased expression of SPINT1 in the skin abrogated matriptase-  
195 induced spontaneous skin squamous cell carcinoma<sup>45</sup>. In zebrafish, reduced expression led to  
196 hyperproliferation of basal keratinocytes<sup>8</sup> and enhanced proliferation of epithelial cells<sup>6</sup>.  
197 Furthermore, SPINT1 deficiency was used to establish a disease model for Skin Cutaneous  
198 Melanoma (SKCM) in zebrafish<sup>4</sup>. In all three studies in zebrafish, skin inflammation was  
199 observed. Third, insertions in introns<sup>8,9</sup> and promoters<sup>6</sup> have caused loss of SPINT1 function.  
200 Together with our genetic localization of the *lf* locus to SPINT1, these lines of evidence make  
201 this gene a very strong candidate for the Lemon Frost phenotype.

202

203 Molecular genetics in reptiles is not well established due to long reproductive cycles and  
204 challenges in laboratory breeding. Early work focused on careful documentation of patterns of  
205 inheritance<sup>18,46</sup>. Molecular studies have examined sequence variants in a candidate  
206 pigmentation gene, melanocortin-1 receptor, and their association with melanic or blanched  
207 phenotypes in different species and ecological niches<sup>47-54</sup>. Recently, CRISPR-Cas9-mediated



208 gene editing was successfully used to mutate the tyrosinase gene in the lizard *Anolis sagrei*<sup>55</sup>.  
209 Although this species is only distantly related to the leopard gecko, this advance offers promise  
210 that targeted studies of the role of SPINT1 mutations in the Lemon Frost phenotype will become  
211 possible.

212

213 White iridophoroma is common in many reptile species<sup>56</sup>, including green iguanas<sup>57</sup>, captive  
214 snakes<sup>58</sup>, bearded dragons<sup>59</sup> and veiled chameleons<sup>60</sup>. The genetic causes of this phenotype in  
215 these species are unknown. Most of our knowledge about molecular and cellular regulation of  
216 iridophores derives from work in zebrafish<sup>27-29,61-71</sup>. Interestingly, few cases of iridophoroma  
217 have been reported in zebrafish<sup>72</sup>. We found that an evolutionarily conserved gene, SPINT1,  
218 regulates the proliferation of white iridophores in the leopard gecko. The tumor suppressor  
219 function of SPINT1 establishes a link between iridophoroma and regulation of white coloration in  
220 reptiles. Our work suggests that cancer genes can play as important a role in iridophores as  
221 they do in melanocytes and melanoma<sup>73</sup>, and that Lemon Frost leopard geckos can serve as a  
222 disease model to study Skin Cutaneous Melanoma.

223

## 224 **Methods**

225

226 Gecko maintenance and experimental procedures

227

228 All activities involving animals included in this manuscript were approved by the University of  
229 California, Los Angeles (UCLA) Institutional Animal Care and Use Committee. Leopard geckos  
230 were acquired from a commercial breeder. Housing conditions at UCLA included: room  
231 temperature of 70-80 F, cage temperature of 72-95 F, room relative humidity between 30-60%,  
232 and a 12:12 hours light cycle. A heating pad was provided at one side of the cage to establish a  
233 temperature gradient. Animals were singly housed in polycarbonate cages with cardboard lines

234 (Techboard<sup>®</sup>) at the bottom, water was provided in bowls inside the cage, and PVC pipe pieces  
235 and plastic plants were offered as environmental enrichment. Geckos were fed 2-6 fresh  
236 crickets and 2-4 mealworms three times per week.

237

238 Geckos were euthanized with an intracoelomic injection of sodium pentobarbital (Euthasol<sup>®</sup>) at  
239 a dose of 100-200 mg/Kg. Immediately after euthanasia, a necropsy was performed, including  
240 external examination, body and organ weighing, gross assessment of normal and abnormal  
241 tissues, and tissue collection for histopathology processing and assessment. Normal and  
242 abnormal tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with  
243 H&E for pathologic evaluation.

244

245 Phenotyping

246

247 Lemon Frost and super Lemon Frost phenotypes were determined according to a list of rules,  
248 based on increased white color of the body, eye, and belly compared to normal wildtype animals  
249 ([http://www.geckosetc.com/lemon\\_frost\\_info.html](http://www.geckosetc.com/lemon_frost_info.html)). Pictures were taken for each animal to  
250 document the phenotype.

251

252 Genotyping

253

254 Genomic DNA was extracted from fresh tail tips with Easy-DNA gDNA purification kit (K180001,  
255 ThermoFisher), or from the saliva with PERFORMAgene (PG-100, DNAGenotek). Genomic  
256 DNA extracted from saliva was further purified with ethanol precipitation before genotyping  
257 assays. DNA libraries for whole genome sequencing were prepared with Nextera DNA Library  
258 Prep Kit (FC-121-1031, Illumina). Libraries for RADseq were prepared according to the

259 procedures of Adapterama III<sup>74</sup> with few modifications. Libraries were sequenced on a HiSeq  
260 3000 (Illumina).

261

262 Only scaffolds larger than 5kb in the draft genome assembly were used as a reference. RADseq  
263 reads and Whole Genome Sequencing (WGS) reads were aligned to the leopard gecko draft  
264 genome<sup>1</sup> with bwa mem<sup>75</sup>. Variants for WGS were identified with GATK<sup>76</sup>. Variants for RADseq  
265 were identified with Stacks<sup>77,78</sup>. All variants were filtered with VCFtools<sup>79</sup>. Only high-quality  
266 variants were used in homozygosity mapping or statistical mapping (DP>=30, GQ>=30).

267

268 Transcriptome sequencing

269

270 Skin tissue samples around 6mm in diameter were taken from the ventral side of the geckos  
271 after anesthetization with 1-5% isoflurane. As tumor tissues are refractory to RNA extraction,  
272 flanking tumor-free tissue samples were taken for homozygous Lemon Frost animals. All  
273 samples were homogenized with TissueRuptor in buffer RLT immediately after collection.  
274 Lysates were immediately frozen on dry ice until all tissues were collected from animals. Then  
275 all lysates were centrifuged for 5 minutes at 13,000 rpm to remove debris. Supernatants were  
276 taken to fresh tubes, and mRNA was extracted according to the procedures of RNeasy Fibrous  
277 Tissue Mini Kit (74704, QIAGEN).

278

279 Libraries of extracted mRNA were prepared with RNA HyperPrep kit (KAPA) and sequenced on  
280 a HiSeq 3000 (Illumina). RNA-seq reads were mapped to the leopard gecko draft genome<sup>1</sup>  
281 using HISAT2 with default parameters. Identification of alternative and differential splicing  
282 events was performed using JuncBase<sup>80</sup>. Gene expression was compared using Sleuth<sup>81</sup> after  
283 RNA transcript abundance was quantified using Kallisto<sup>82</sup>.

284

285 Pathology

286

287 Complete postmortem examination was performed, and representative tissue samples were  
288 obtained. All tissues obtained at necropsy were preserved in 10% neutral-buffered formalin  
289 solution for up to 5 days before being processed and embedded in paraffin. All tissues were  
290 sectioned at 5  $\mu$ m, and routinely stained with Hematoxylin and Eosin.

291

292 Statistical Mapping

293

294 Biallelic markers with minor allele frequency of less than 5% and with fewer than 10 individuals  
295 called as homozygous for both the reference and alternative alleles were excluded from  
296 mapping and kinship matrix construction. A kinship matrix was calculated using the function  
297 *A.mat* with default parameters from the rrBLUP<sup>83</sup> R package. Phenotype was encoded as 0 for  
298 wild type, 1 for Lemon Frost, and 2 for super Lemon Frost. Association statistics between this  
299 phenotype vector and marker genotypes were computed using the function *gwas2* in the NAM<sup>84</sup>  
300 R package using a linear mixed model with a random effect of kinship to control for population  
301 structure. The effective number of tests was computed to be 141.1 based on the procedure of  
302 Galwey et al<sup>85</sup>. A family-wise error rate significance threshold was calculated as 0.01/141.1 or  
303  $p < 7.09e-5$ .

304

305 Homozygosity Mapping

306

307 Pooled animals and Mr. Frosty were sequenced to ~30x coverage on a HiSeq 3000 (Illumina).  
308 Variants were identified with GATK and filtered with VCFtools. Biallelic heterozygous variants  
309 from Mr. Frosty, including indels, were used as markers to localize the Lemon Frost mutation.  
310 Allele ratios (AF) were calculated by dividing the read count of alternative alleles by the sum of

311 the counts of reference alleles and alternative alleles. Variants closely linked to the Lemon Frost  
312 mutation are expected to have AF between 0.4 and 0.6 in the Lemon Frost pool and in Mr.  
313 Frosty, AF > 0.85 in the super Lemon Frost pool, and AF < 0.15 in the wildtype pool. The  
314 number of variants meeting these criteria was counted for every 10kb genome interval. The  
315 fraction of such variants among all variants heterozygous in Mr. Frosty within the interval was  
316 then calculated. Intervals with fewer than 5 variants were excluded because they could not  
317 provide statistically meaningful results.

318

### 319 Transmission Electron Microscopy

320

321 Dissected skin tissues were fixed in 2.5% glutaraldehyde and 4% formaldehyde in 0.1 M sodium  
322 cacodylate buffer overnight at 4 °C. After being washed in PBS, samples were post-fixed in 1%  
323 osmium tetroxide in 0.1M sodium cacodylate, and dehydrated through a graded series of  
324 ethanol concentrations. After infiltration with Eponate 12 resin, the samples were embedded in  
325 fresh Eponate 12 resin and polymerized at 60°C for 48 hours. Ultrathin sections of 70 nm  
326 thickness were prepared, placed on formvar-coated copper grids, and stained with uranyl  
327 acetate and Reynolds' lead citrate. The grids were examined using a JEOL 100CX transmission  
328 electron microscope at 60 kV, and images were captured by an AMT digital camera (Advanced  
329 Microscopy Techniques Corporation, model XR611).

330

### 331 **Acknowledgement**

332 We thank Aaron Miller, Jasmine Gonzalez, Kendall Placido and James Walter for their  
333 assistance in gecko DNA collection and phenotyping. We thank members of the Kruglyak lab for  
334 helpful feedbacks on the project, and Giancarlo Bruni, Stefan Zdraljevic, Eyal Ben-David and  
335 Olga Schubert for helpful comments on the manuscript. We thank Chunni Zhu (Electron

336 Microscopy Core Facility, UCLA Brain Research Institute) for her assistance in TEM sample  
337 processing and imaging. We thank Jonathan Eggenschwiler for helpful discussions.

338

339 This work is supported by the Howard Hughes Medical Institute (LK) and the Helen Hay  
340 Whitney Foundation (LG).

341

## 342 **Figure legends**

343

344 **Fig 1 The Lemon Frost mutant of the common leopard gecko, *Eublepharis macularius*.** (A)  
345 wild type; (B) heterozygous mutant; (C) homozygous mutant, with red arrow pointing to the eye  
346 lid; (D) blizzard mutant with minimal color; (E) Lemon Frost mutation (*lf*) on the blizzard  
347 background; (F-H) segregation of the *lf* allele. Lemon Frost (LF) denotes heterozygotes for the  
348 mutation; super LF denotes homozygotes for the mutation. All proportions are consistent with  
349 expectations for single-locus Mendelian inheritance (chi-square test  $p > 0.1$ ).

350

351 **Fig 2 Tumor growth and metastasis in the Lemon Frost mutant.** Designations are  
352 homozygous mutant (*lf/lf*); heterozygous mutant (*lf/+*); wild type (*+/+*). (A) tumors in ventral skin;  
353 (B) thick layers of white tumor cells (*lf/lf*) vs. normal white cells (*+/+*); (C) outgrowth of white  
354 tumor cells (*lf/+*); (D) metastasis of white tumor cells in the liver and oral cavity. Red arrows:  
355 white colored tumor cells. Arrowhead in B: normal white cells.

356

357 **Fig 3 Localization of the Lemon Frost mutation.** (A) p-value for association with white color  
358 and (B) linkage disequilibrium for 28 markers syntenic to chicken chromosome 15 (red, ordered  
359 by synteny), 4 markers syntenic to chromosome 5 (cyan), and 16 markers without synteny  
360 information (green). (C) A schematic of the region showing synteny and gene annotation. (D)  
361 Fraction of markers showing expected allele frequency pattern in pools, plotted for 10kb  
362 windows along scaffold 996. The four windows with the highest fraction are marked by asterisks  
363 and span the location of the gene SPINT1. Windows with fewer than 5 variants were not plotted  
364 (dashed red lines). (E) Genome-wide distribution of the fraction of markers showing expected  
365 allele frequency pattern in pools for all 10 kb windows. The 4 highest windows on scaffold 996  
366 (red arrows) marked in D are among the 6 highest windows in the entire genome.

367

368 **Fig 4 The *lemon frost* allele in a backcross.** (A) We genotyped 7 progeny with the Lemon  
369 Frost phenotype and 6 wild type progeny from the third generation of a backcross of Mr. Frosty  
370 to the Sunburst line for markers in the SPINT1 region and observed a consistent inheritance  
371 pattern. (B) Sequencing chromatogram of a heterozygous animal (*lf/+*) at an insertion marker.  
372 (C) Sequencing chromatogram of a homozygous animal (*+/+*) at the same insertion marker.

373

374 **SupFig 1 Coloration and pattern diversity of the common leopard gecko, *Eublepharis***  
375 ***macularius*.** (A) wild type; (B) black night; (C) variant of black night; (D) granite snow; (E) gem  
376 snow; (F) white knight; (G) sunburst tangerine; (H-I) variants of sunburst tangerine; (J) red  
377 stripes; (K) bold stripes; (L) rainbow.

378

379 **SupFig 2 Breeding pedigree of the Lemon Frost mutation.** Mr. Frosty, the original carrier of  
380 the spontaneous Lemon Frost mutation, was bred to 12 female geckos from different genetic

381 backgrounds. F1s carrying the *lf* allele were bred among themselves or back to their female  
382 parent, producing the second generation of animals heterozygous or homozygous for the *lf*  
383 allele. Blue: *lf/lf*; green: *lf/+*; red: *+/+*. Dashed line: same individual/line.

384

385 **SupFig 3 Histopathology of skin tumors.** (A) Thick layers of white tumor tissue (star)  
386 infiltrating white skin (arrow). (B) Skin biopsies organized and fixed in a paper roll for sectioning.  
387 (C) H&E staining of the skin sections. Arrow: skin; star: infiltrated tumor mass. (D) H&E staining  
388 of the skin sections showing normal skin cells and neoplastic cells (star). Neoplastic cells have  
389 eccentric and condensed nuclei.

390

391 **SupFig 4 Potential metastasis of iridophoroma.** (A) In normal skin, cell nuclei are oval and  
392 perpendicular to the skin surface. In Lemon Frost skin, cell nuclei are flat, elongated and parallel  
393 to the skin, reminiscent of epithelial-to-mesenchymal transition. (B) Iridophoroma in the liver,  
394 stained dark in H&E sections. In dark field imaging, iridophores are bright white. Such  
395 iridophores invade blood vessels in the tissue (red arrows). (C) In TEM imaging, white tumor  
396 skins in super LF are filled with abundant iridophores with excessive brightly reflective crystals  
397 (Tumor). In normal skin, iridophores are much fewer and have less crystals (Normal).

398

399 **SupFig 5 SPINT1 expression in gecko skin.** SPINT1 mRNA reads from transcriptome  
400 sequencing were aligned to the genome and visualized in IGV. Top 3 rows show samples from  
401 homozygous mutants. Bottom 3 rows show samples from wild type geckos. Skin tissue adjacent  
402 to the tumors was used in the mutants. Peaks mark SPINT1 exons. The last exon on the right is  
403 transcribed together with the 3'UTR.

404

405

## 406 **References**

407

- 408 1 Xiong, Z. *et al.* Draft genome of the leopard gecko, *Eublepharis macularius*. *GigaScience*  
409 **5**, doi:10.1186/s13742-016-0151-4 (2016).
- 410 2 Kataoka, H., Kawaguchi, M., Fukushima, T. & Shimomura, T. Hepatocyte growth factor  
411 activator inhibitors (HAI-1 and HAI-2): Emerging key players in epithelial integrity and  
412 cancer. *Pathol Int* **68**, 145-158, doi:10.1111/pin.12647 (2018).
- 413 3 Shimomura, T. *et al.* Hepatocyte growth factor activator inhibitor, a novel Kunitz-type  
414 serine protease inhibitor. *J Biol Chem* **272**, 6370-6376, doi:10.1074/jbc.272.10.6370  
415 (1997).
- 416 4 Gomez-Abenza, E. *et al.* Zebrafish modeling reveals that SPINT1 regulates the  
417 aggressiveness of skin cutaneous melanoma and its crosstalk with tumor immune  
418 microenvironment. *J Exp Clin Cancer Res* **38**, 405, doi:10.1186/s13046-019-1389-3  
419 (2019).
- 420 5 Kawaguchi, M. *et al.* Membrane-bound serine protease inhibitor HAI-1 is required for  
421 maintenance of intestinal epithelial integrity. *Am J Pathol* **179**, 1815-1826,  
422 doi:10.1016/j.ajpath.2011.06.038 (2011).
- 423 6 Mathias, J. R. *et al.* Live imaging of chronic inflammation caused by mutation of  
424 zebrafish Hai1. *J Cell Sci* **120**, 3372-3383, doi:10.1242/jcs.009159 (2007).
- 425 7 Kawaguchi, M. *et al.* Inhibition of nuclear factor-kappaB signaling suppresses Spint1-  
426 deletion-induced tumor susceptibility in the ApcMin/+ model. *Oncotarget* **7**, 68614-  
427 68622, doi:10.18632/oncotarget.11863 (2016).

- 428 8 Carney, T. J. *et al.* Inactivation of serine protease Matriptase1a by its inhibitor Hai1 is  
429 required for epithelial integrity of the zebrafish epidermis. *Development* **134**, 3461-3471,  
430 doi:10.1242/dev.004556 (2007).
- 431 9 Fan, B. *et al.* Hepatocyte growth factor activator inhibitor-1 (HAI-1) is essential for the  
432 integrity of basement membranes in the developing placental labyrinth. *Dev Biol* **303**,  
433 222-230, doi:10.1016/j.ydbio.2006.11.005 (2007).
- 434 10 Hoshiko, S. *et al.* Hepatocyte growth factor activator inhibitor type 1 is a suppressor of  
435 intestinal tumorigenesis. *Cancer Res* **73**, 2659-2670, doi:10.1158/0008-5472.CAN-12-  
436 3337 (2013).
- 437 11 Tanaka, H. *et al.* Hepatocyte growth factor activator inhibitor type 1 (HAI-1) is required  
438 for branching morphogenesis in the chorioallantoic placenta. *Mol Cell Biol* **25**, 5687-  
439 5698, doi:10.1128/MCB.25.13.5687-5698.2005 (2005).
- 440 12 Nagaike, K. *et al.* Defect of hepatocyte growth factor activator inhibitor type 1/serine  
441 protease inhibitor, Kunitz type 1 (Hai-1/Spint1) leads to ichthyosis-like condition and  
442 abnormal hair development in mice. *Am J Pathol* **173**, 1464-1475,  
443 doi:10.2353/ajpath.2008.071142 (2008).
- 444 13 Sakugawa, C. *et al.* Prognostic significance of hepatocyte growth factor activator  
445 inhibitor type 1 (HAI-1) immunoreactivity in pancreatic ductal adenocarcinoma. *BMC*  
446 *Res Notes* **10**, 674, doi:10.1186/s13104-017-3014-x (2017).
- 447 14 Baba, T. *et al.* Loss of membrane-bound serine protease inhibitor HAI-1 induces oral  
448 squamous cell carcinoma cells' invasiveness. *J Pathol* **228**, 181-192,  
449 doi:10.1002/path.3993 (2012).
- 450 15 Ye, J. *et al.* Loss of hepatocyte growth factor activator inhibitor type 1 participates in  
451 metastatic spreading of human pancreatic cancer cells in a mouse orthotopic  
452 transplantation model. *Cancer Sci* **105**, 44-51, doi:10.1111/cas.12306 (2014).
- 453 16 Kataoka, H. *et al.* Distribution of hepatocyte growth factor activator inhibitor type 1  
454 (HAI-1) in human tissues. Cellular surface localization of HAI-1 in simple columnar  
455 epithelium and its modulated expression in injured and regenerative tissues. *J*  
456 *Histochem Cytochem* **47**, 673-682, doi:10.1177/002215549904700509 (1999).
- 457 17 Fujii, R. in *International Review of Cytology* Vol. 143 (eds Kwang W. Jeon, Martin  
458 Friedlander, & Jonathan Jarvik) 191-255 (Academic Press, 1993).
- 459 18 Olsson, M., Stuart-Fox, D. & Ballen, C. Genetics and evolution of colour patterns in  
460 reptiles. *Seminars in Cell & Developmental Biology* **24**, 529-541, doi:  
461 10.1016/j.semcdb.2013.04.001 (2013).
- 462 19 Cuthill, I. C. *et al.* The biology of color. *Science* **357**, doi:10.1126/science.aan0221 (2017).
- 463 20 Shawkey, M. D. & D'Alba, L. Interactions between colour-producing mechanisms and  
464 their effects on the integumentary colour palette. *Philos Trans R Soc Lond B Biol Sci* **372**,  
465 doi:10.1098/rstb.2016.0536 (2017).
- 466 21 Thayer, R. C., Allen, F. I. & Patel, N. H. Structural color in *Junonia* butterflies evolves by  
467 tuning scale lamina thickness. *Elife* **9**, doi:10.7554/eLife.52187 (2020).
- 468 22 Kelsh, R. N., Harris, M. L., Colanesi, S. & Erickson, C. A. Stripes and belly-spots -- a review  
469 of pigment cell morphogenesis in vertebrates. *Semin Cell Dev Biol* **20**, 90-104,  
470 doi:10.1016/j.semcdb.2008.10.001 (2009).



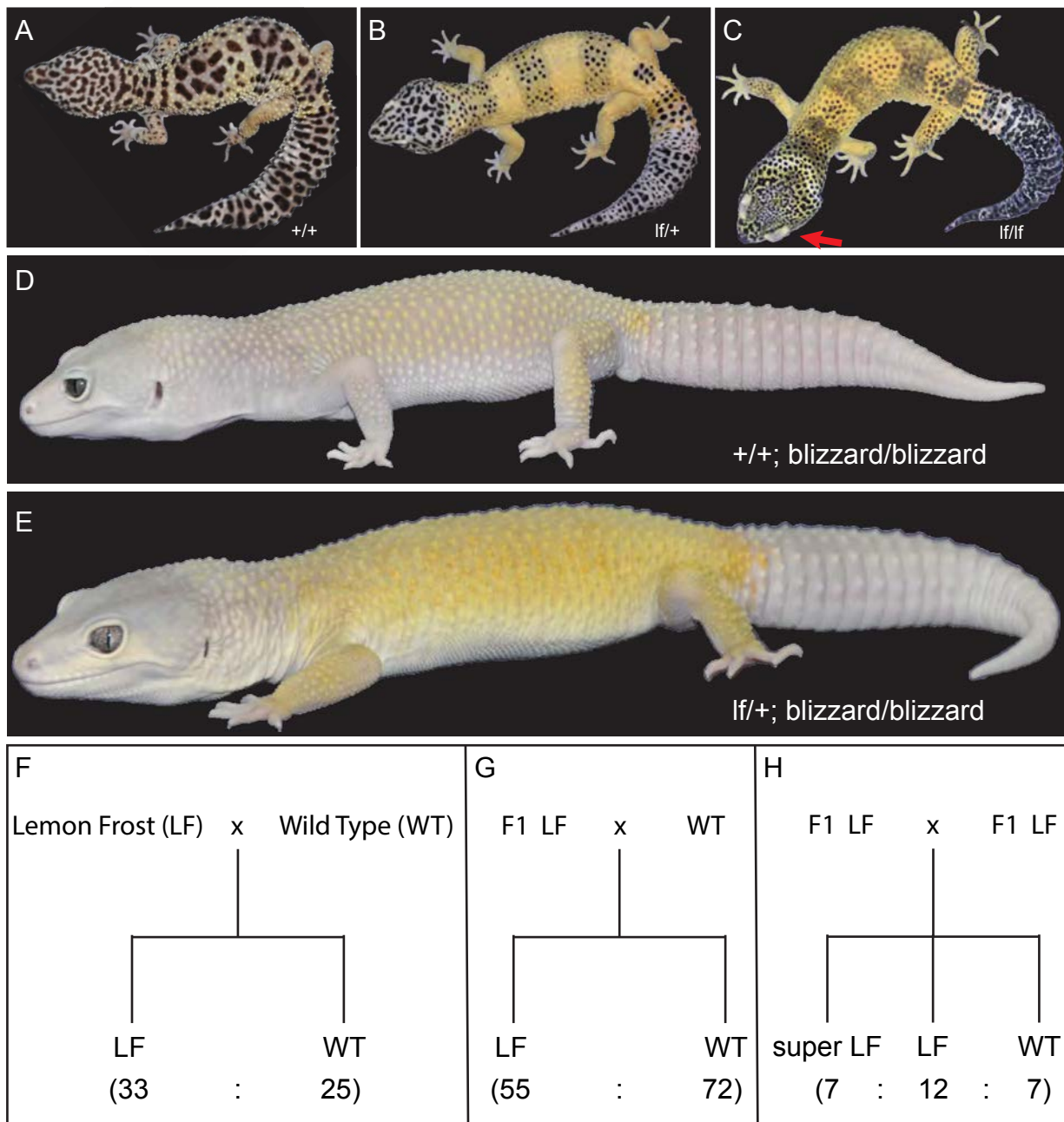
- 471 23 Parichy, D. M. & Spiewak, J. E. Origins of adult pigmentation: diversity in pigment stem  
472 cell lineages and implications for pattern evolution. *Pigment Cell Melanoma Res* **28**, 31-  
473 50, doi:10.1111/pcmr.12332 (2015).
- 474 24 Nordlund, J. J., Abdel-Malek, Z. A., Boissy, R. E. & Rheins, L. A. Pigment Cell Biology: An  
475 Historical Review. *Journal of Investigative Dermatology* **92**, S53-S60, doi:  
476 10.1038/jid.1989.33 (1989).
- 477 25 Nordlund, J. J. *et al.* *The Pigmentary System: Physiology and Pathophysiology*. 2nd edn,  
478 (Wiley, 2008).
- 479 26 Goda, M. & Fujii, R. The Blue Coloration of the Common Surgeonfish, *Paracanthurus*  
480 *hepatus*-II. Color Revelation and Color Changes. *Zoolog Sci* **15**, 323-333,  
481 doi:10.2108/zsj.15.323 (1998).
- 482 27 Frohnhofer, H. G., Krauss, J., Maischein, H. M. & Nusslein-Volhard, C. Iridophores and  
483 their interactions with other chromatophores are required for stripe formation in  
484 zebrafish. *Development* **140**, 2997-3007, doi:10.1242/dev.096719 (2013).
- 485 28 Salis, P. *et al.* Developmental and comparative transcriptomic identification of  
486 iridophore contribution to white barring in clownfish. *Pigment Cell Melanoma Res* **32**,  
487 391-402, doi:10.1111/pcmr.12766 (2019).
- 488 29 Krauss, J. *et al.* Endothelin signalling in iridophore development and stripe pattern  
489 formation of zebrafish. *Biol Open* **3**, 503-509, doi:10.1242/bio.20148441 (2014).
- 490 30 Kaelin, C. B. *et al.* Specifying and sustaining pigmentation patterns in domestic and wild  
491 cats. *Science* **337**, 1536-1541, doi:10.1126/science.1220893 (2012).
- 492 31 Wise, P. A. D., Vickaryous, M. K. & Russell, A. P. An Embryonic Staging Table for In Ovo  
493 Development of *Eublepharis macularius*, the Leopard Gecko. *The Anatomical Record* **292**,  
494 1198-1212, doi:10.1002/ar.20945 (2009).
- 495 32 McLean, K. E. & Vickaryous, M. K. A novel amniote model of epimorphic regeneration:  
496 the leopard gecko, *Eublepharis macularius*. *BMC Developmental Biology* **11**, 50,  
497 doi:10.1186/1471-213X-11-50 (2011).
- 498 33 Delorme, S. L., Lungu, I. M. & Vickaryous, M. K. Scar-Free Wound Healing and  
499 Regeneration Following Tail Loss in the Leopard Gecko, *Eublepharis macularius*. *The*  
500 *Anatomical Record* **295**, 1575-1595, doi:10.1002/ar.22490 (2012).
- 501 34 Szydłowski, P. *et al.* Iridophoroma associated with the Lemon Frost colour morph of the  
502 leopard gecko (*Eublepharis macularius*). *Scientific Reports* **10**, 5734,  
503 doi:10.1038/s41598-020-62828-9 (2020).
- 504 35 Bagnara, J. T. in *International Review of Cytology* Vol. 20 (eds G. H. Bourne & J. F.  
505 Danielli) 173-205 (Academic Press, 1966).
- 506 36 Denefle, J. P. & Lechaire, J. P. Localization of pigment cells in cultured frog skin. *Am J*  
507 *Anat* **188**, 212-220, doi:10.1002/aja.1001880210 (1990).
- 508 37 DeMartini, D. G., Krogstad, D. V. & Morse, D. E. Membrane invaginations facilitate  
509 reversible water flux driving tunable iridescence in a dynamic biophotonic system. *Proc*  
510 *Natl Acad Sci U S A* **110**, 2552-2556, doi:10.1073/pnas.1217260110 (2013).
- 511 38 Aramaki, T. & Kondo, S. Method for disarranging the pigment pattern of zebrafish by  
512 optogenetics. *Developmental Biology* **460**, 12-19, doi: 10.1016/j.ydbio.2018.12.019  
513 (2020).

- 514 39 Morrison, R. L. & Frost-Mason, S. K. Ultrastructural analysis of iridophore  
515 organellogenesis in a lizard, *Sceloporus graciosus* (Reptilia: Phrynosomatidae). *J Morphol*  
516 **209**, 229-239, doi:10.1002/jmor.1052090209 (1991).
- 517 40 Hillier, L. W. *et al.* Sequence and comparative analysis of the chicken genome provide  
518 unique perspectives on vertebrate evolution. *Nature* **432**, 695-716,  
519 doi:10.1038/nature03154 (2004).
- 520 41 Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409**,  
521 860-921, doi:10.1038/35057062 (2001).
- 522 42 Cheng, H., Fukushima, T., Takahashi, N., Tanaka, H. & Kataoka, H. Hepatocyte growth  
523 factor activator inhibitor type 1 regulates epithelial to mesenchymal transition through  
524 membrane-bound serine proteinases. *Cancer Res* **69**, 1828-1835, doi:10.1158/0008-  
525 5472.CAN-08-3728 (2009).
- 526 43 Kataoka, H. *et al.* Evaluation of hepatocyte growth factor activator inhibitor expression  
527 in normal and malignant colonic mucosa. *Cancer Letters* **128**, 219-227, doi:  
528 10.1016/S0304-3835(98)00067-6 (1998).
- 529 44 Koivuniemi, R. *et al.* Hepatocyte growth factor activator inhibitor-1 is induced by bone  
530 morphogenetic proteins and regulates proliferation and cell fate of neural progenitor  
531 cells. *PLoS One* **8**, e56117, doi:10.1371/journal.pone.0056117 (2013).
- 532 45 List, K. *et al.* Deregulated matriptase causes ras-independent multistage carcinogenesis  
533 and promotes ras-mediated malignant transformation. *Genes Dev* **19**, 1934-1950,  
534 doi:10.1101/gad.1300705 (2005).
- 535 46 Olsson, M. *et al.* Mating system variation and morph fluctuations in a polymorphic lizard.  
536 *Molecular Ecology* **16**, 5307-5315, doi: 10.1111/j.1365-294X.2007.03578.x (2007).
- 537 47 Micheletti, S., Parra, E. & Routman, E. J. Adaptive Color Polymorphism and Unusually  
538 High Local Genetic Diversity in the Side-Blotched Lizard, *Uta stansburiana*. *PLOS ONE* **7**,  
539 e47694, doi:10.1371/journal.pone.0047694 (2012).
- 540 48 Rosenblum, E. B., Hoekstra, H. E. & Nachman, M. W. ADAPTIVE REPTILE COLOR  
541 VARIATION AND THE EVOLUTION OF THE MC1R GENE. *Evolution* **58**, 1794-1808, doi:  
542 10.1111/j.0014-3820.2004.tb00462.x (2004).
- 543 49 Nunes, V. L., Miraldo, A., Beaumont, M. A., Butlin, R. K. & Paulo, O. S. Association of  
544 Mc1r variants with ecologically relevant phenotypes in the European ocellated lizard,  
545 *Lacerta lepida*. *Journal of Evolutionary Biology* **24**, 2289-2298, doi: 10.1111/j.1420-  
546 9101.2011.02359.x (2011).
- 547 50 Fulgione, D., Lega, C., Trapanese, M. & Buglione, M. Genetic factors implied in melanin-  
548 based coloration of the Italian wall lizard. *Journal of Zoology* **296**, 278-285, doi:  
549 10.1111/jzo.12242 (2015).
- 550 51 Rosenblum, E. B., Römler, H., Schöneberg, T. & Hoekstra, H. E. Molecular and  
551 functional basis of phenotypic convergence in white lizards at White Sands. *Proceedings*  
552 *of the National Academy of Sciences* **107**, 2113, doi:10.1073/pnas.0911042107 (2010).
- 553 52 Laurent, S. *et al.* The population genomics of rapid adaptation: disentangling signatures  
554 of selection and demography in white sands lizards. *Molecular Ecology* **25**, 306-323, doi:  
555 10.1111/mec.13385 (2016).

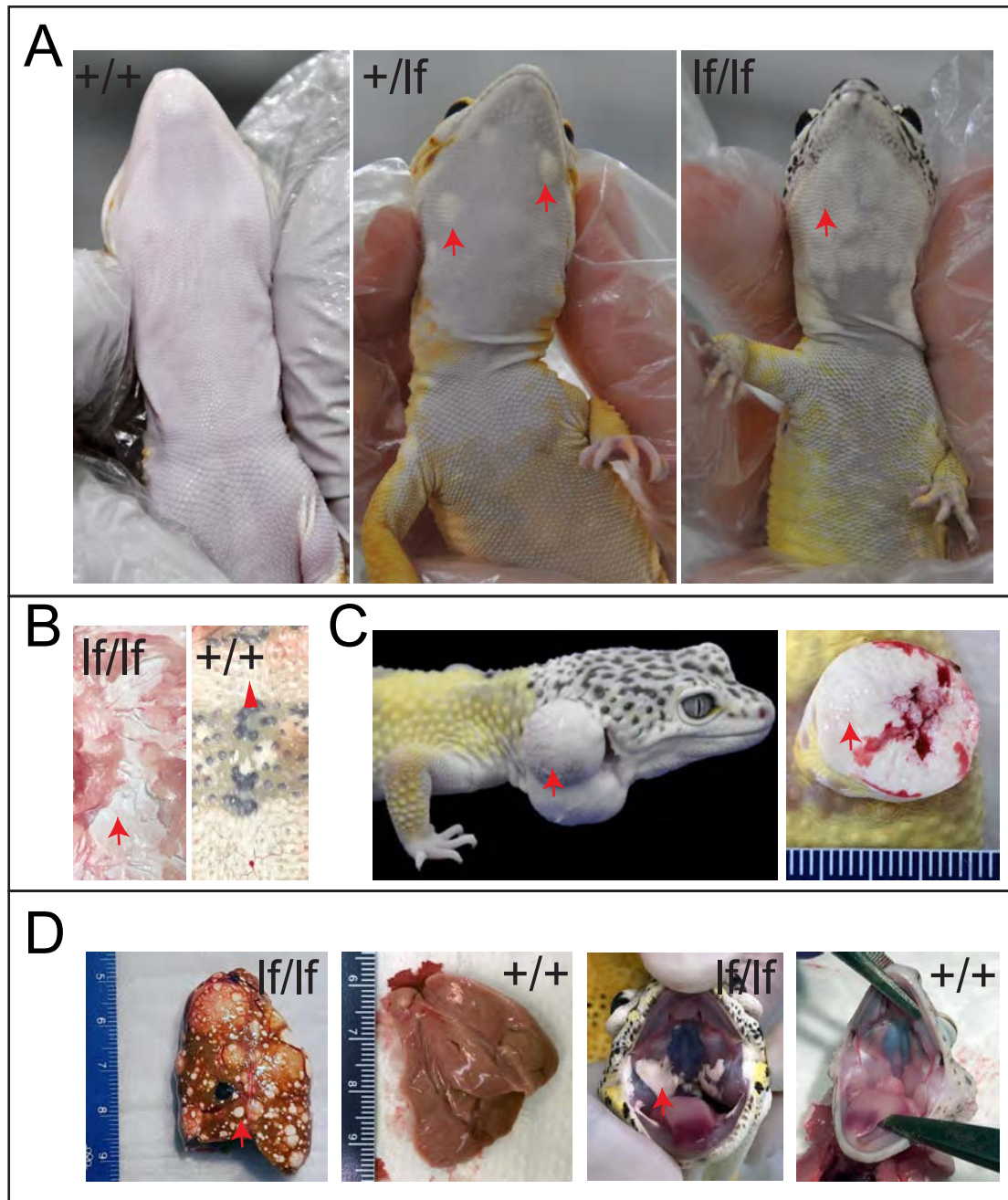
- 556 53 Corso, J., Gonçalves, G. L. & Freitas, T. R. O. d. Sequence variation in the melanocortin-1  
557 receptor (MC1R) pigmentation gene and its role in the cryptic coloration of two South  
558 American sand lizards. *Genetics and Molecular Biology* **35**, 81-87 (2012).
- 559 54 Jin, Y. *et al.* Dorsal Pigmentation and Its Association with Functional Variation in MC1R in  
560 a Lizard from Different Elevations on the Qinghai-Tibetan Plateau. *Genome Biology and*  
561 *Evolution*, doi:10.1093/gbe/evaa225 (2020).
- 562 55 Rasys, A. M. *et al.* CRISPR-Cas9 Gene Editing in Lizards through Microinjection of  
563 Unfertilized Oocytes. *Cell Rep* **28**, 2288-2292 e2283, doi:10.1016/j.celrep.2019.07.089  
564 (2019).
- 565 56 Heckers, K. O., Aupperle, H., Schmidt, V. & Pees, M. Melanophoromas and  
566 iridophoromas in reptiles. *J Comp Pathol* **146**, 258-268, doi:10.1016/j.jcpa.2011.07.003  
567 (2012).
- 568 57 Rousselet, E. *et al.* Cutaneous iridophoroma in a Green iguana (*Iguana iguana*). *Vet Clin*  
569 *Pathol* **46**, 625-628, doi:10.1111/vcp.12536 (2017).
- 570 58 Munoz-Gutierrez, J. F., Garner, M. M. & Kiupel, M. Cutaneous Chromatophoromas in  
571 Captive Snakes. *Vet Pathol* **53**, 1213-1219, doi:10.1177/0300985816644302 (2016).
- 572 59 de Brot, S., Sydler, T., Nufer, L. & Ruetten, M. Histologic, Immunohistochemical, and  
573 Electron Microscopic Characterization of a Malignant Iridophoroma in a Dwarf Bearded  
574 Dragon (*Pogona Henrylawsoni*). *J Zoo Wildl Med* **46**, 583-587, doi:10.1638/2013-0113.1  
575 (2015).
- 576 60 Bronson, E., Pereira, M., Sanchez, C. & Murray, S. Iridophoroma in a Veiled Chameleon,  
577 *Chamaeleo calyptratus*. *Journal of Herpetological Medicine and Surgery* **16**, 58-60,  
578 doi:10.5818/1529-9651.16.3.58 (2006).
- 579 61 Patterson, L. B. & Parichy, D. M. Interactions with iridophores and the tissue  
580 environment required for patterning melanophores and xanthophores during zebrafish  
581 adult pigment stripe formation. *PLoS Genet* **9**, e1003561,  
582 doi:10.1371/journal.pgen.1003561 (2013).
- 583 62 Volkening, A. & Sandstede, B. Iridophores as a source of robustness in zebrafish stripes  
584 and variability in *Danio* patterns. *Nat Commun* **9**, 3231, doi:10.1038/s41467-018-05629-  
585 z (2018).
- 586 63 Hirata, M., Nakamura, K. & Kondo, S. Pigment cell distributions in different tissues of the  
587 zebrafish, with special reference to the striped pigment pattern. *Dev Dyn* **234**, 293-300,  
588 doi:10.1002/dvdy.20513 (2005).
- 589 64 Hirata, M., Nakamura, K., Kanemaru, T., Shibata, Y. & Kondo, S. Pigment cell  
590 organization in the hypodermis of zebrafish. *Dev Dyn* **227**, 497-503,  
591 doi:10.1002/dvdy.10334 (2003).
- 592 65 Singh, A. P., Schach, U. & Nusslein-Volhard, C. Proliferation, dispersal and patterned  
593 aggregation of iridophores in the skin prefigure striped colouration of zebrafish. *Nat Cell*  
594 *Biol* **16**, 607-614, doi:10.1038/ncb2955 (2014).
- 595 66 Krauss, J. *et al.* transparent, a gene affecting stripe formation in Zebrafish, encodes the  
596 mitochondrial protein Mpv17 that is required for iridophore survival. *Biol Open* **2**, 703-  
597 710, doi:10.1242/bio.20135132 (2013).

- 598 67 Fadeev, A., Krauss, J., Singh, A. P. & Nusslein-Volhard, C. Zebrafish Leucocyte tyrosine  
599 kinase controls iridophore establishment, proliferation and survival. *Pigment Cell*  
600 *Melanoma Res* **29**, 284-296, doi:10.1111/pcmr.12454 (2016).
- 601 68 Singh, A. P. & Nusslein-Volhard, C. Zebrafish stripes as a model for vertebrate colour  
602 pattern formation. *Curr Biol* **25**, R81-R92, doi:10.1016/j.cub.2014.11.013 (2015).
- 603 69 Cooper, C. D. *et al.* Protein Kinase A Signaling Inhibits Iridophore Differentiation in  
604 Zebrafish. *J Dev Biol* **6**, doi:10.3390/jdb6040023 (2018).
- 605 70 Irion, U. & Nusslein-Volhard, C. The identification of genes involved in the evolution of  
606 color patterns in fish. *Curr Opin Genet Dev* **57**, 31-38, doi:10.1016/j.gde.2019.07.002  
607 (2019).
- 608 71 Lewis, V. M. *et al.* Fate plasticity and reprogramming in genetically distinct populations  
609 of Danio leucophores. *Proc Natl Acad Sci U S A* **116**, 11806-11811,  
610 doi:10.1073/pnas.1901021116 (2019).
- 611 72 Masahito, P., Ishikawa, T. & Sugano, H. Pigment cells and pigment cell tumors in fish. *J*  
612 *Invest Dermatol* **92**, 266S-270S, doi:10.1111/1523-1747.ep13076602 (1989).
- 613 73 Yang, K., Oak, A. S. W., Slominski, R. M., Brozyna, A. A. & Slominski, A. T. Current  
614 Molecular Markers of Melanoma and Treatment Targets. *Int J Mol Sci* **21**,  
615 doi:10.3390/ijms21103535 (2020).
- 616 74 Bayona-Vasquez, N. J. *et al.* Adapterama III: Quadruple-indexed, double/triple-enzyme  
617 RADseq libraries (2RAD/3RAD). *PeerJ* **7**, e7724, doi:10.7717/peerj.7724 (2019).
- 618 75 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler  
619 transform. *Bioinformatics* **25**, 1754-1760, doi:10.1093/bioinformatics/btp324 (2009).
- 620 76 McKenna, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for analyzing  
621 next-generation DNA sequencing data. *Genome Res* **20**, 1297-1303,  
622 doi:10.1101/gr.107524.110 (2010).
- 623 77 Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A. & Cresko, W. A. Stacks: an  
624 analysis tool set for population genomics. *Mol Ecol* **22**, 3124-3140,  
625 doi:10.1111/mec.12354 (2013).
- 626 78 Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J. H. Stacks:  
627 building and genotyping Loci de novo from short-read sequences. *G3 (Bethesda)* **1**, 171-  
628 182, doi:10.1534/g3.111.000240 (2011).
- 629 79 Danecek, P. *et al.* The variant call format and VCFtools. *Bioinformatics* **27**, 2156-2158,  
630 doi:10.1093/bioinformatics/btr330 (2011).
- 631 80 Brooks, A. N. *et al.* Conservation of an RNA regulatory map between Drosophila and  
632 mammals. *Genome Res* **21**, 193-202, doi:10.1101/gr.108662.110 (2011).
- 633 81 Pimentel, H., Bray, N. L., Puente, S., Melsted, P. & Pachter, L. Differential analysis of  
634 RNA-seq incorporating quantification uncertainty. *Nat Methods* **14**, 687-690,  
635 doi:10.1038/nmeth.4324 (2017).
- 636 82 Bray, N. L., Pimentel, H., Melsted, P. & Pachter, L. Near-optimal probabilistic RNA-seq  
637 quantification. *Nat Biotechnol* **34**, 525-527, doi:10.1038/nbt.3519 (2016).
- 638 83 Endelman, J. B. Ridge Regression and Other Kernels for Genomic Selection with R  
639 Package rrBLUP. *The Plant Genome* **4**, 250-255, doi:10.3835/plantgenome2011.08.0024  
640 (2011).

- 641 84 Xavier, A., Xu, S., Muir, W. M. & Rainey, K. M. NAM: association studies in multiple  
642 populations. *Bioinformatics* **31**, 3862-3864, doi:10.1093/bioinformatics/btv448 (2015).  
643 85 Galwey, N. W. A new measure of the effective number of tests, a practical tool for  
644 comparing families of non-independent significance tests. *Genet Epidemiol* **33**, 559-568,  
645 doi:10.1002/gepi.20408 (2009).  
646

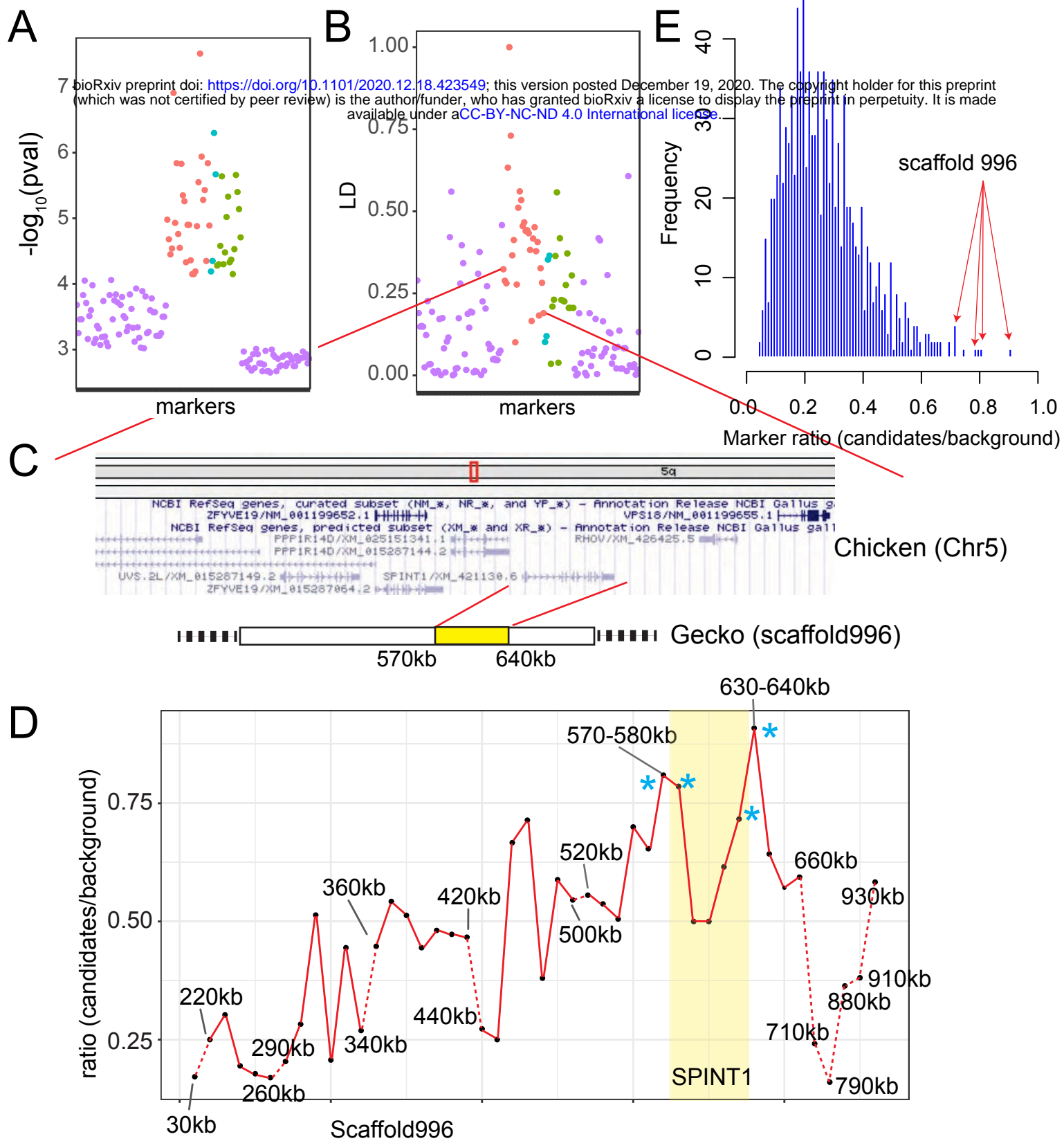


**Fig 1 The Lemon Frost mutant of the common leopard gecko, *Eublepharis macularius*.** (A) wild type; (B) heterozygous mutant; (C) homozygous mutant, with red arrow pointing to the eye lid; (D) blizzard mutant with minimal color; (E) Lemon Frost mutation (lf) on the blizzard background; (F-H) segregation of the lf allele. Lemon Frost (LF) denotes heterozygotes for the mutation; super LF denotes homozygotes for the mutation. All proportions are consistent with expectations for single-locus Mendelian inheritance (chi-square test  $p > 0.1$ ).



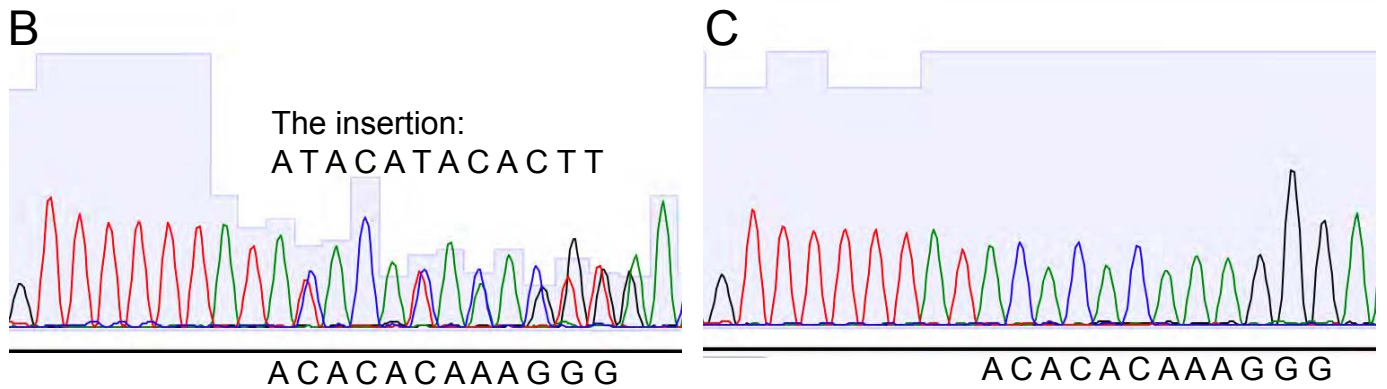
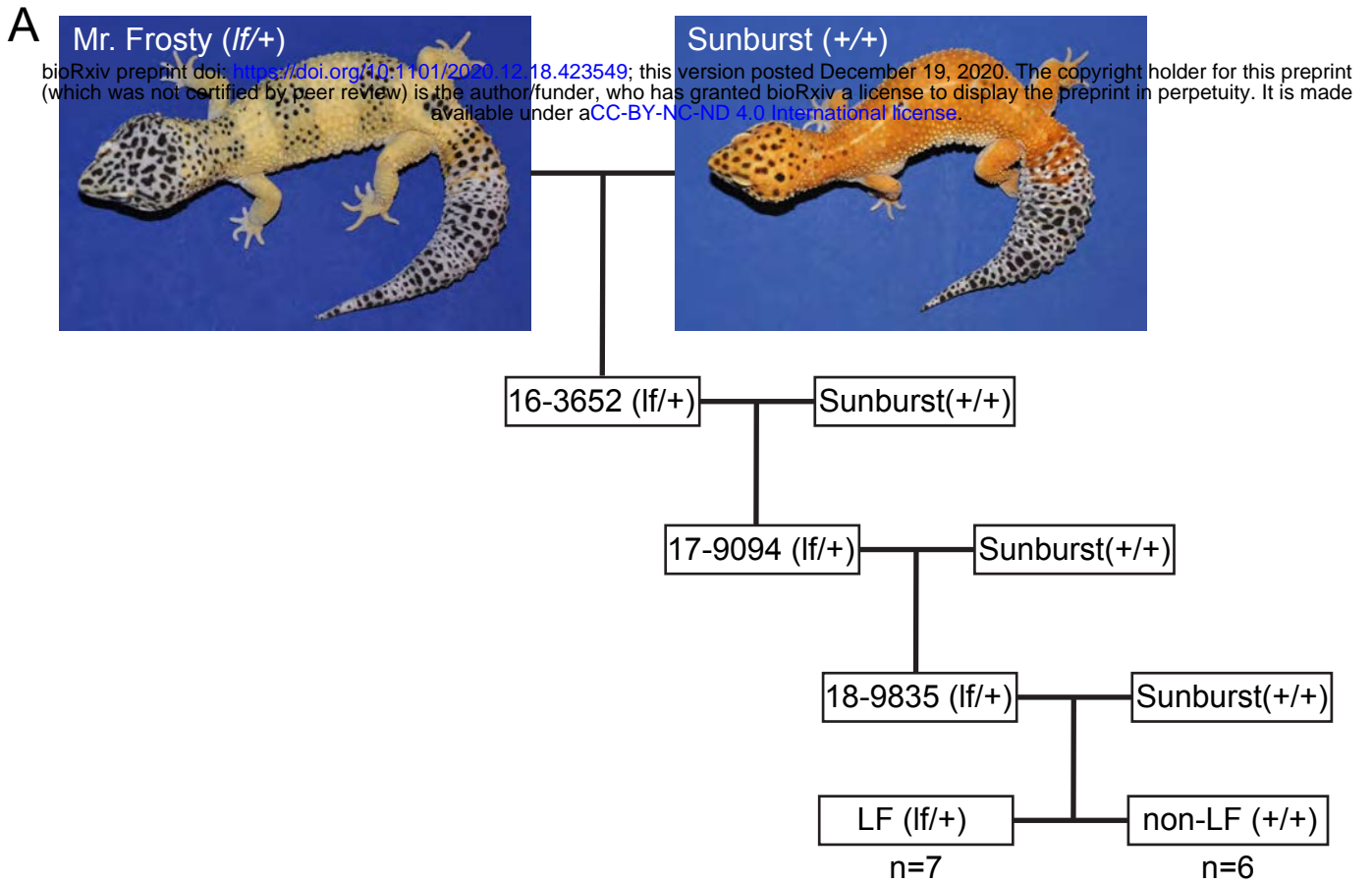
**Fig 2 Tumor growth and metastasis in the Lemon Frost mutant.**

Designations are homozygous mutant ( $lf/lf$ ); heterozygous mutant ( $lf/lf$ );  $\Delta$  wild type ( $+/+$ ). (A) tumors in ventral skin; (B) thick layers of white tumor cells ( $lf/lf$ ) vs. normal white cells ( $+/+$ ); (C) outgrowth of white tumor cells ( $lf/+$ ); (D) metastasis of white tumor cells in the liver and oral cavity. Red arrows: white colored tumor cells. Arrowhead in B: normal white cells.

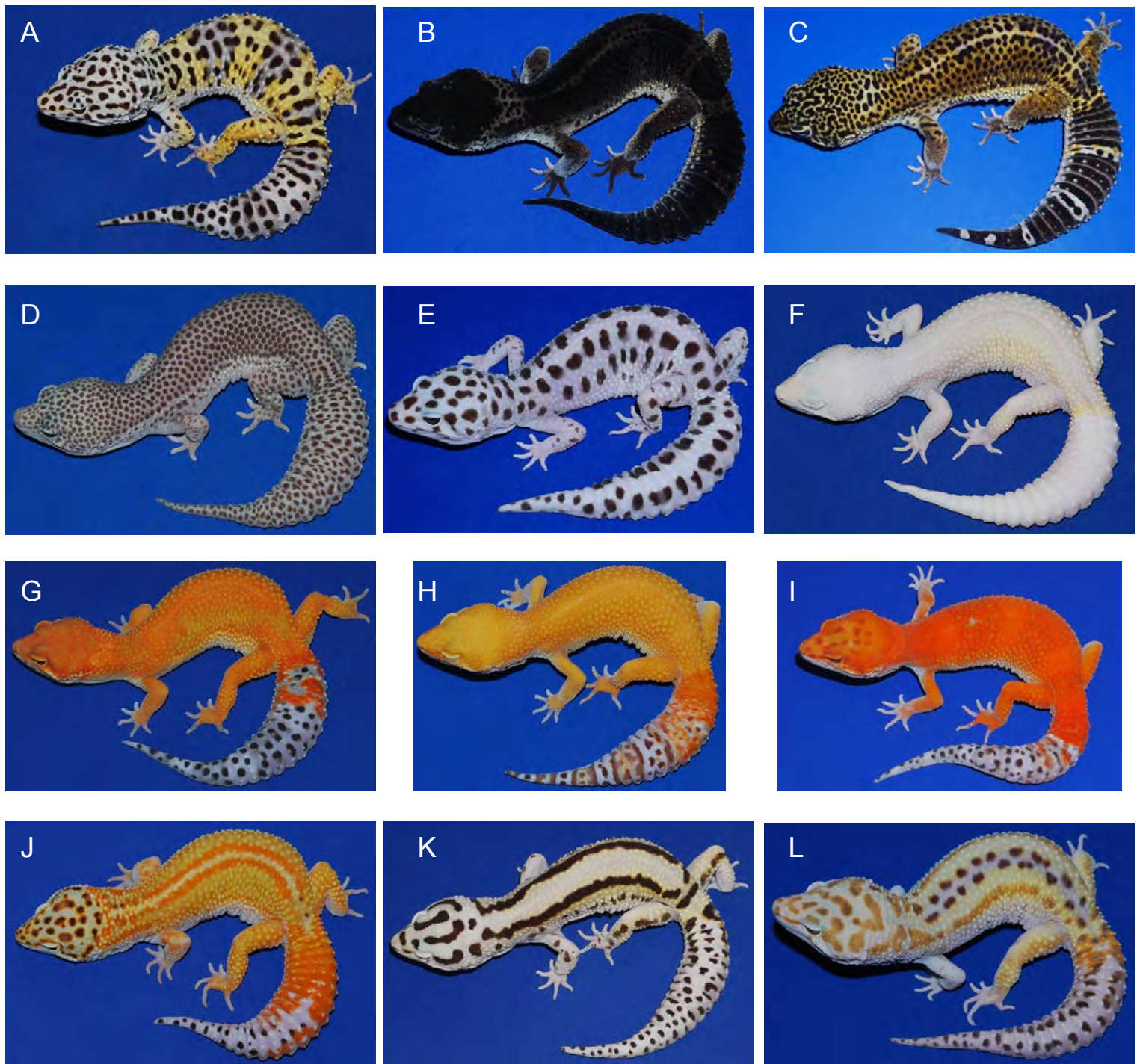


**Fig 3 Localization of the Lemon Frost mutation.** (A) p-value for association with white color and (B) linkage disequilibrium for 28 markers syntenic to chicken chromosome 1 (red, ordered by synteny), 4 markers syntenic to chromosome 5 (cyan), and 16 markers without synteny information (green). (C) A schematic of the region showing synteny and gene annotation. (D) Fraction of markers showing expected allele frequency pattern in pools, plotted for 10kb windows along scaffold 99. The four windows with the highest fraction are marked by asterisks and span the location of the gene SPINT1. Windows with fewer than 5 variants were not plotted (dashed red lines). (E) Genome-wide distribution of the fraction of markers showing expected allele frequency pattern in pools for all 10 kb windows. The 4 highest windows on scaffold 99 (red arrows) marked in D are among the 6 highest windows in the entire genome.

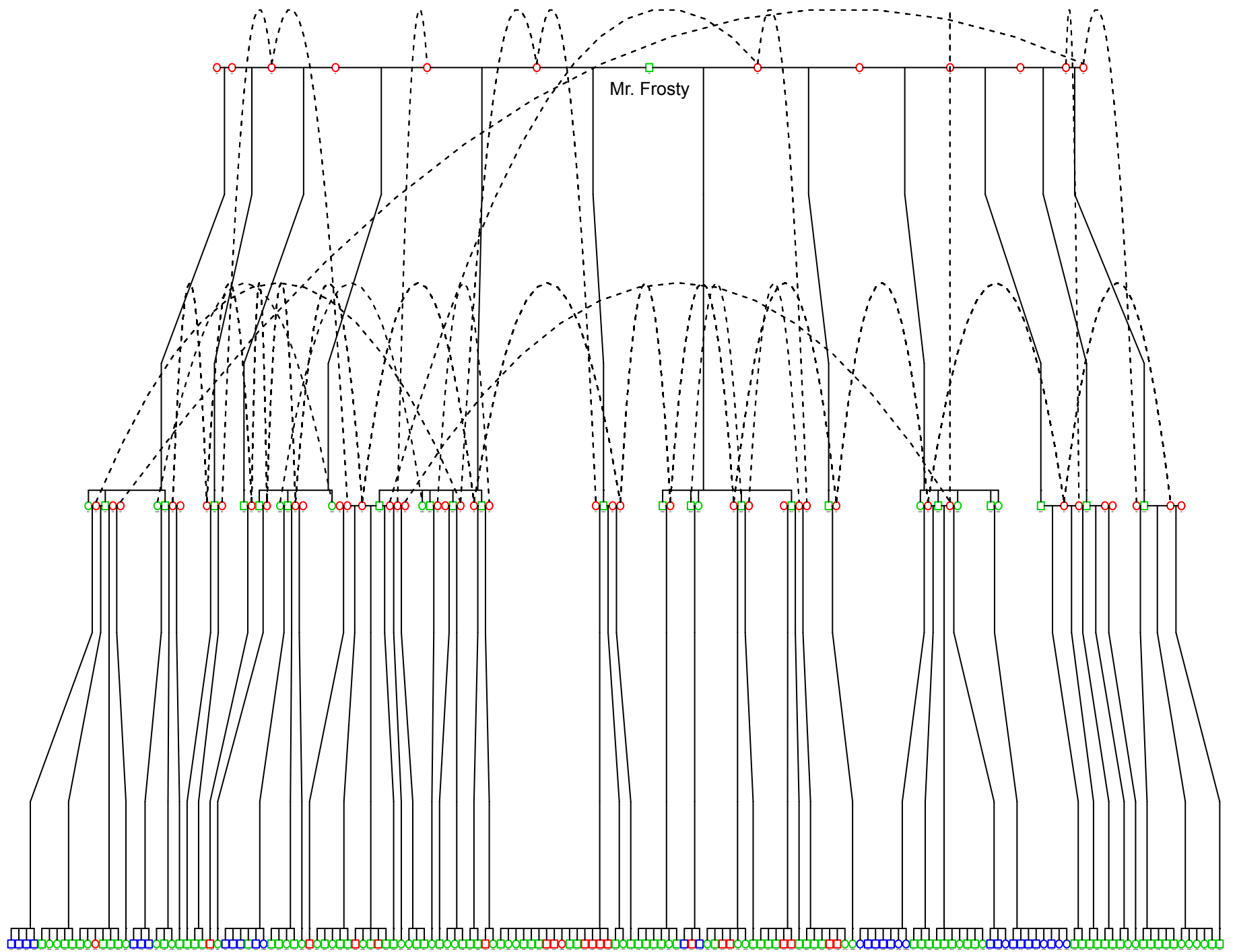




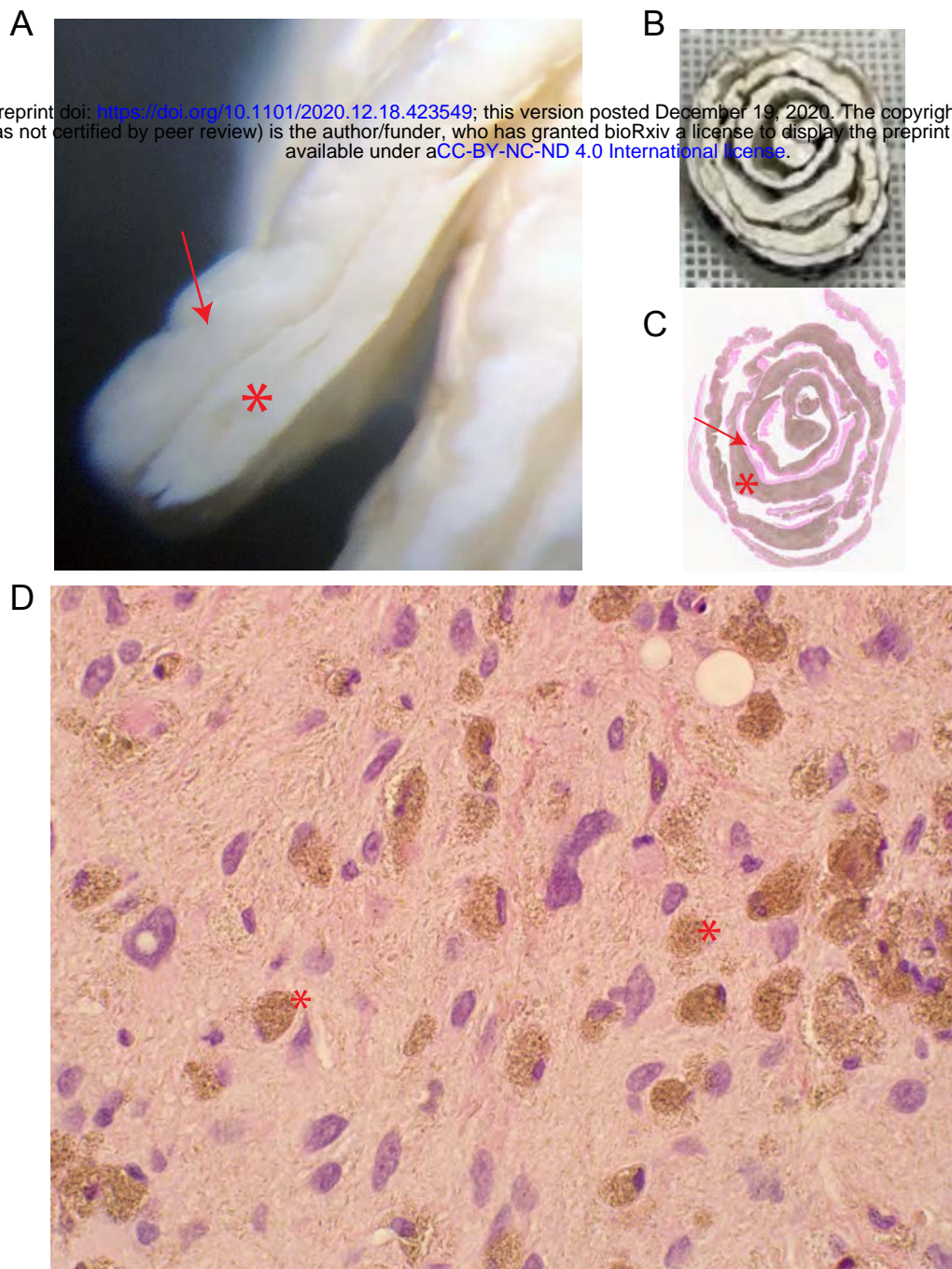
**Fig The *lemon frost* allele in a backcross.** (A) We genotyped 7 progeny with the Lemon Frost phenotype and 6 wild type progeny from the third generation of a backcross of Mr. Frosty to the Sunburst line for markers in the SPINT1 region and observed a consistent inheritance pattern. (B) Sequencing chromatogram of a heterozygous animal (*lf/+*) at an insertion marker. (C) Sequencing chromatogram of a homozygous animal (*+/+*) at the same insertion marker.



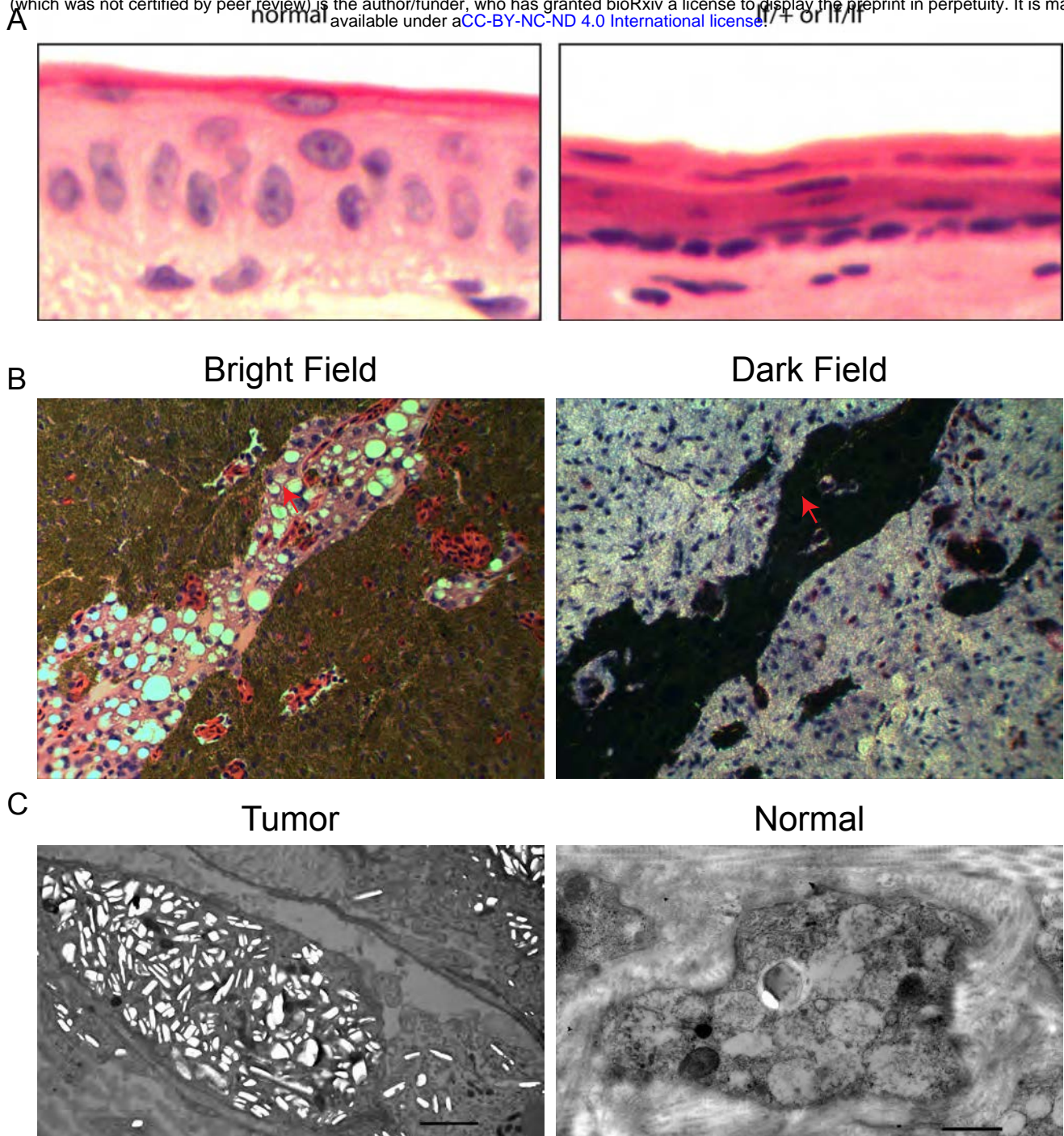
**SupFig 1** Coloration and pattern diversity of the common leopard gecko, *Eublepharis macularius*. (A) wild type; (B) black night; (C) variant of black night; (D) granite snow; (E) gem snow; (F) white knight; (G) sunburst tangerine; (H-I) variants of sunburst tangerine; (J) red stripes; (K) bold stripes; (L) rainbow.



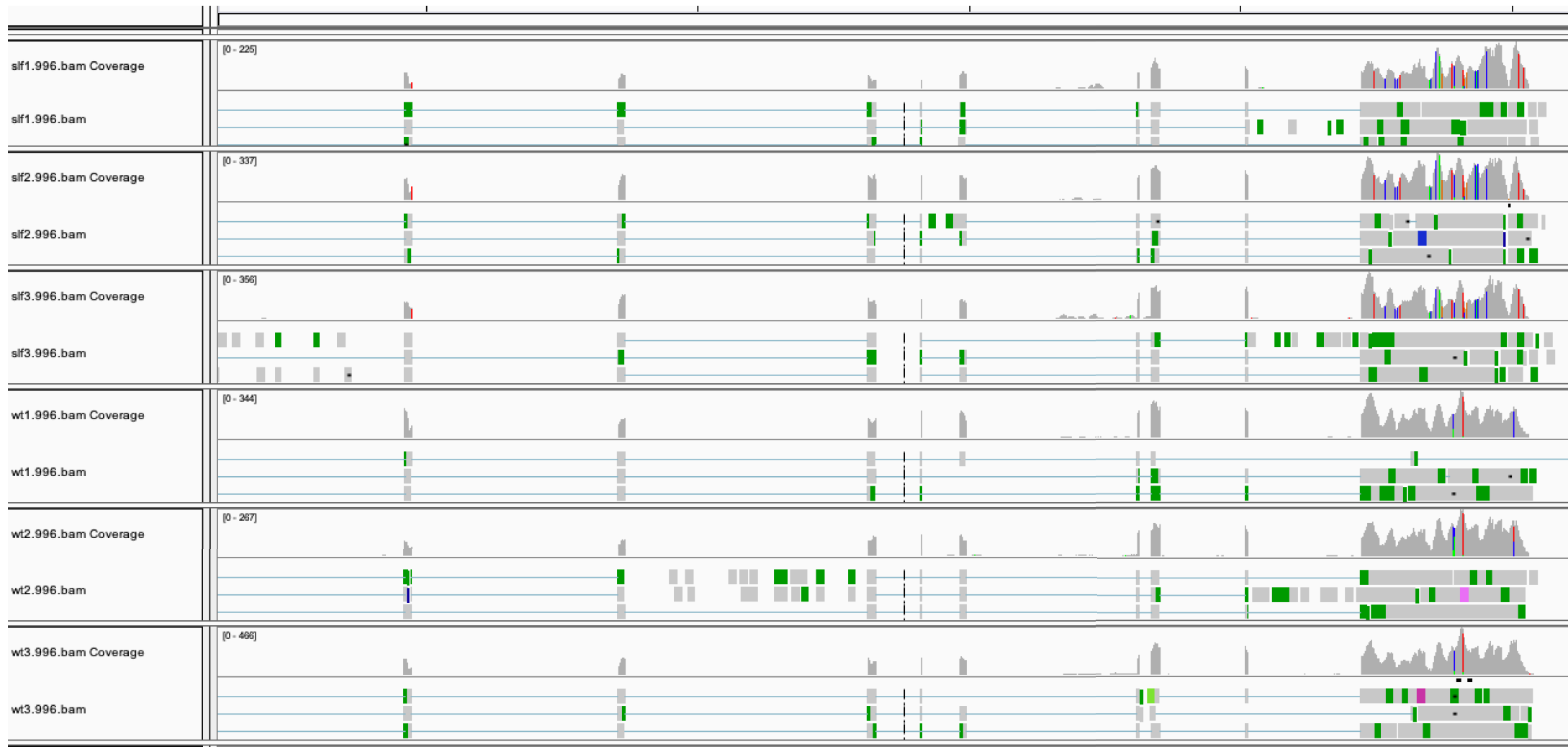
**SupFig 2 Breeding pedigree of the Lemon Frost mutation.** Mr. Frosty, the original carrier of the spontaneous Lemon Frost mutation, was bred to 12 female geckos from different genetic backgrounds. F1s carrying the *lf* allele were bred among themselves or back to their female parent, producing the second generation of animals heterozygous or homozygous for the *lf* allele. Blue: *lf/lf*; green: *lf/+*; red: *+/+*. Dashed line:



**SupFig 3 Histopathology of skin tumors.** (A) Thick layers of white tumor tissue (star) infiltrating white skin (arrow). (B) Skin biopsies organized and fixed in a paper roll for sectioning. (C) H&E staining of the skin sections. Arrow: skin; star: infiltrated tumor mass. (D) H&E staining of the skin sections showing normal skin cells and neoplastic cells (star). Neoplastic cells have eccentric and condensed nuclei.



**SupFig 4 Potential metastasis of iridophoroma.** (A) In normal skin, cell nuclei are oval and perpendicular to the skin surface. In Lemon Frost skin, cell nuclei are flat, elongated and parallel to the skin, reminiscent of epithelial-to-mesenchymal transition. (B) iridophoroma in the liver, stained dark in H&E sections. In dark field imaging, iridophores are bright white. Such iridophores invade blood vessels in the tissue (red arrows). (C) In TEM imaging, white tumor skins in super LF are filled with abundant iridophores with excessive brightly reflective crystals (Tumor). In normal skin, iridophores are much fewer and have less crystals (Normal).



**SupFig 5 SPINT1 expression in gecko skin.** SPINT1 mRNA reads from transcriptome sequencing were aligned to the genome and visualized in IGV. Top 3 rows show samples from homozygous mutants. Bottom rows show samples from wild type geckos. Skin tissue adjacent to the tumors was used in the mutants. Peaks mark SPINT1 exons. The last exon on the right is transcribed together with the 3'UTR.