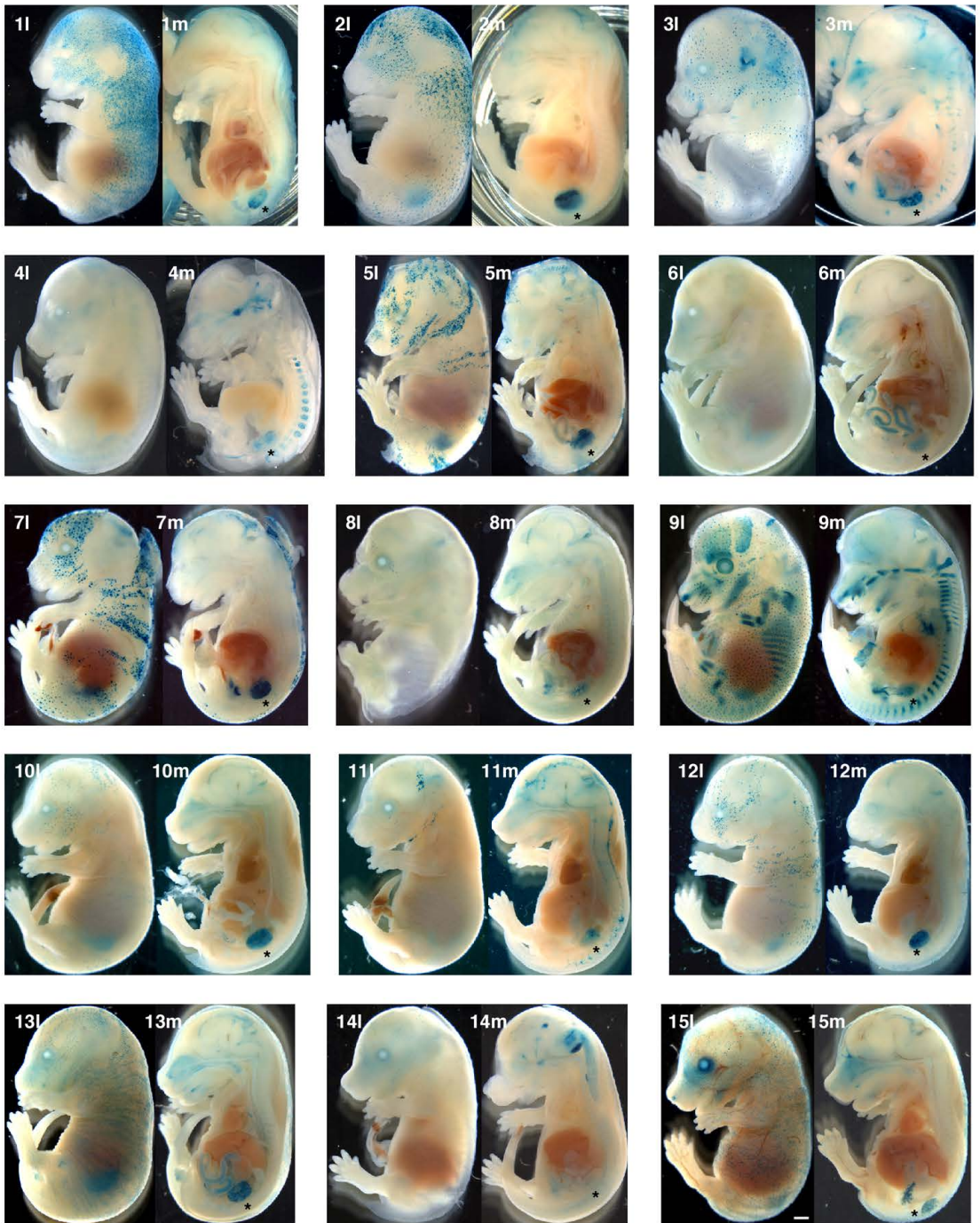
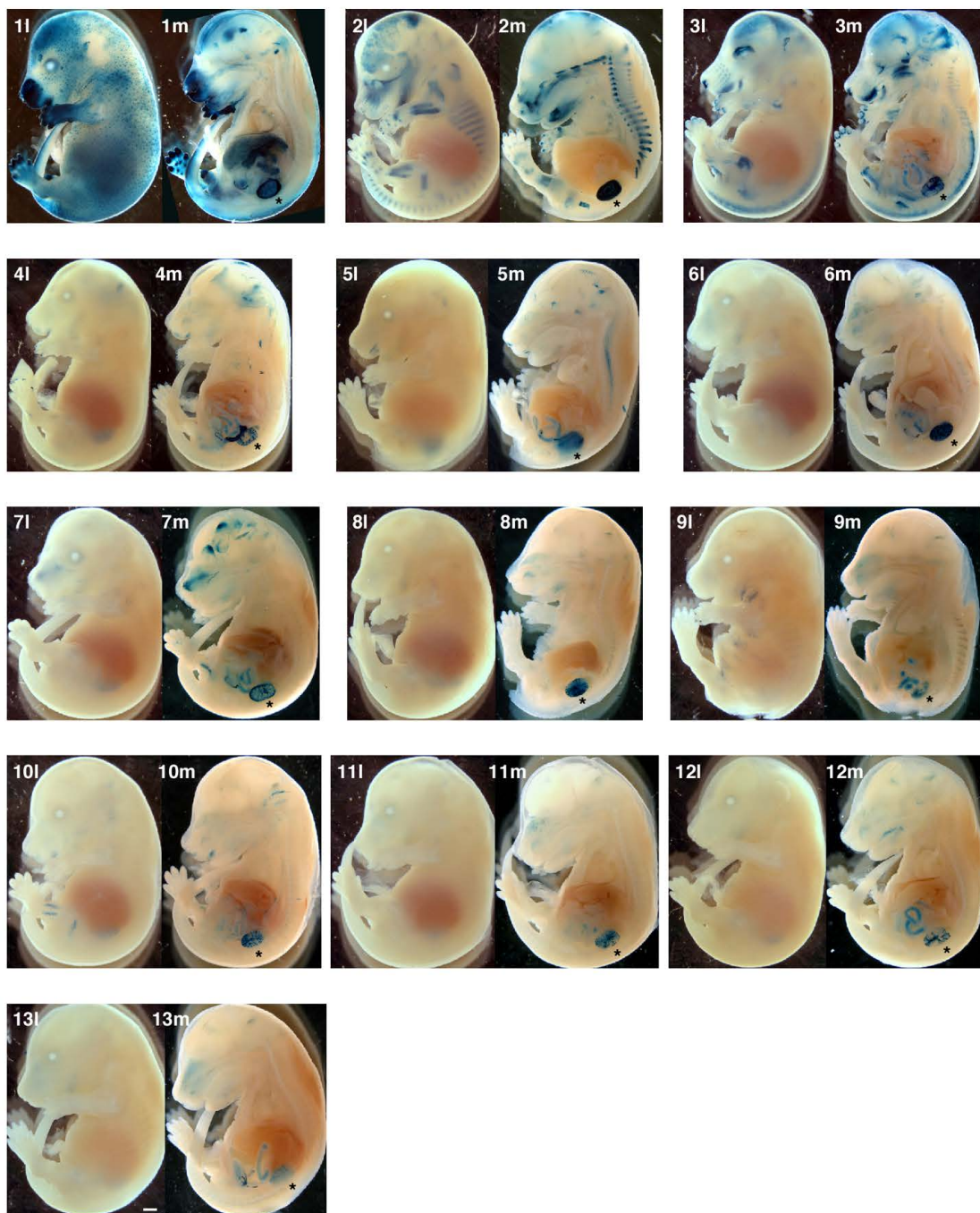
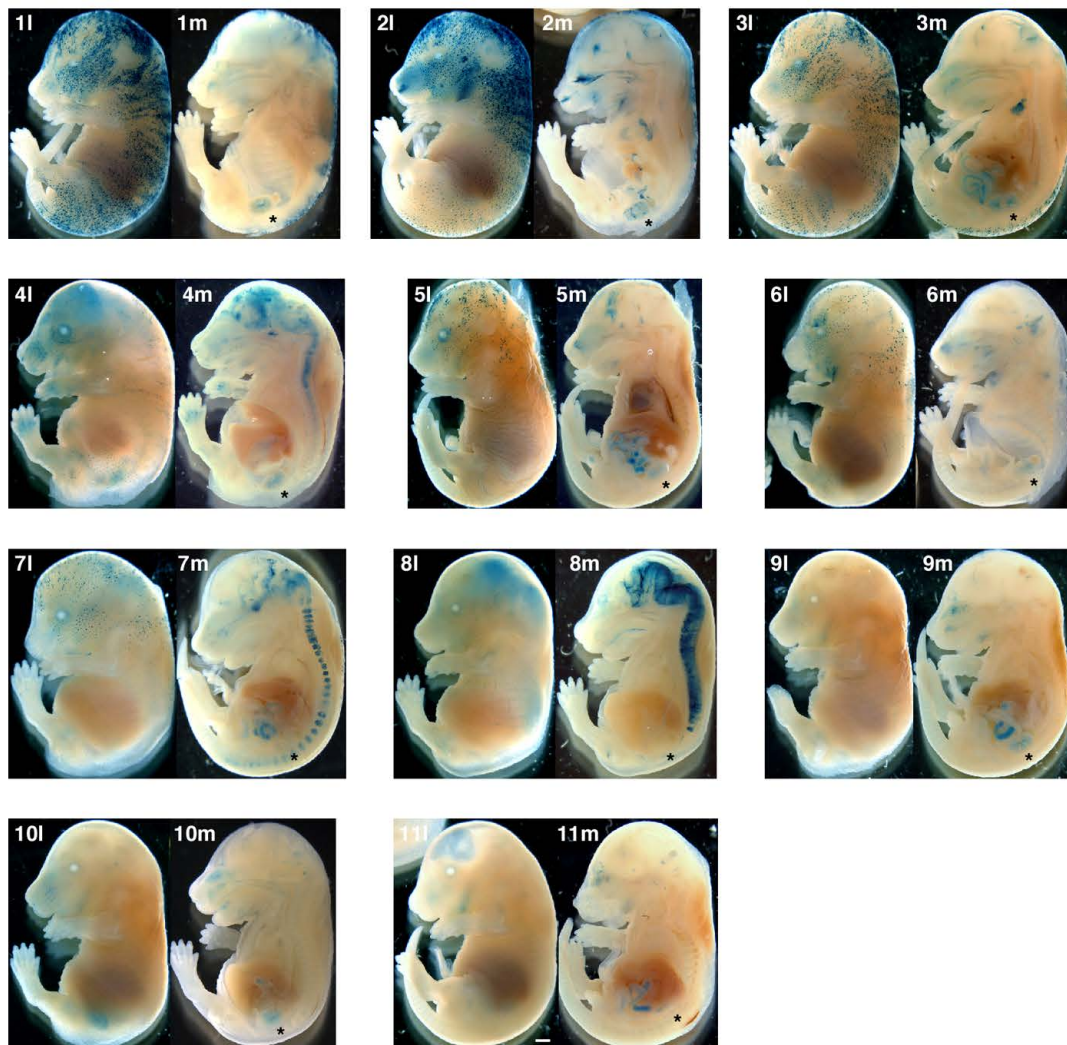




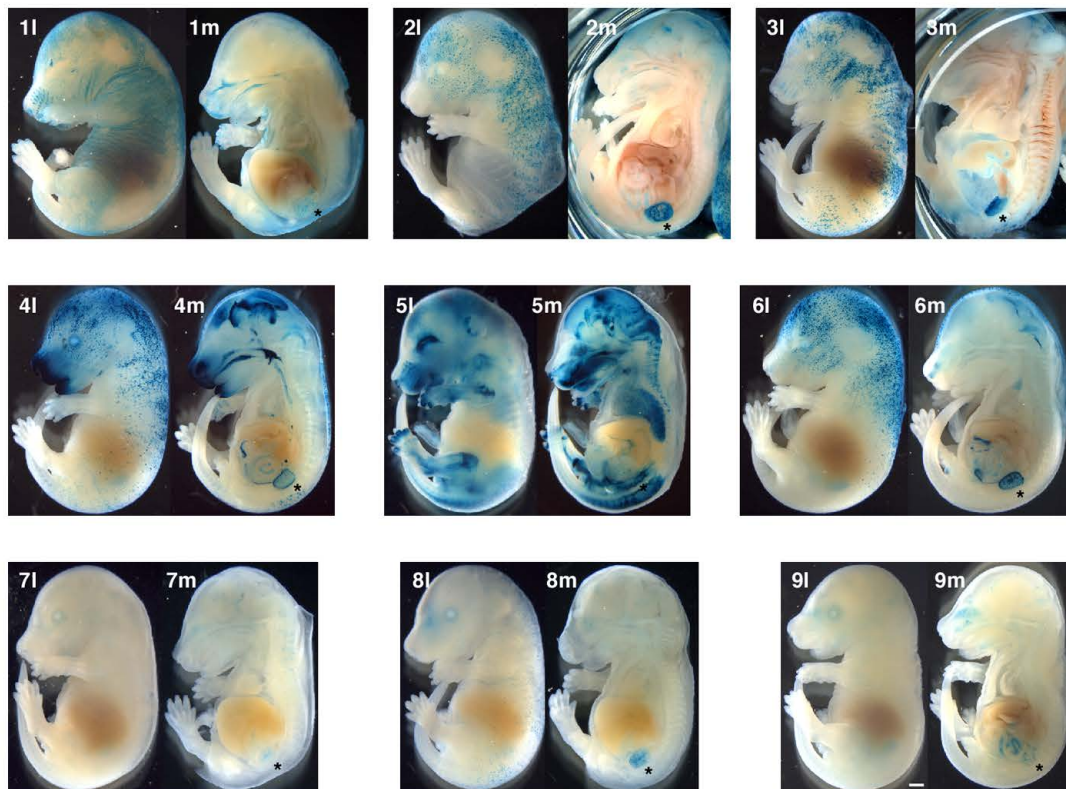
Supplementary Figure 1. H2 transgenic embryos. Fifteen transgenic embryos produced by pronuclear injection of the 6.7 kb H2 plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to show both external lateral (l) and internal medial (m) expression patterns. The skin ($n = 13$) and kidney ($n = 14$) were consistent sites of expression. The asterisk denotes the position of the kidney in the internal images. Scale bar, 1 mm.



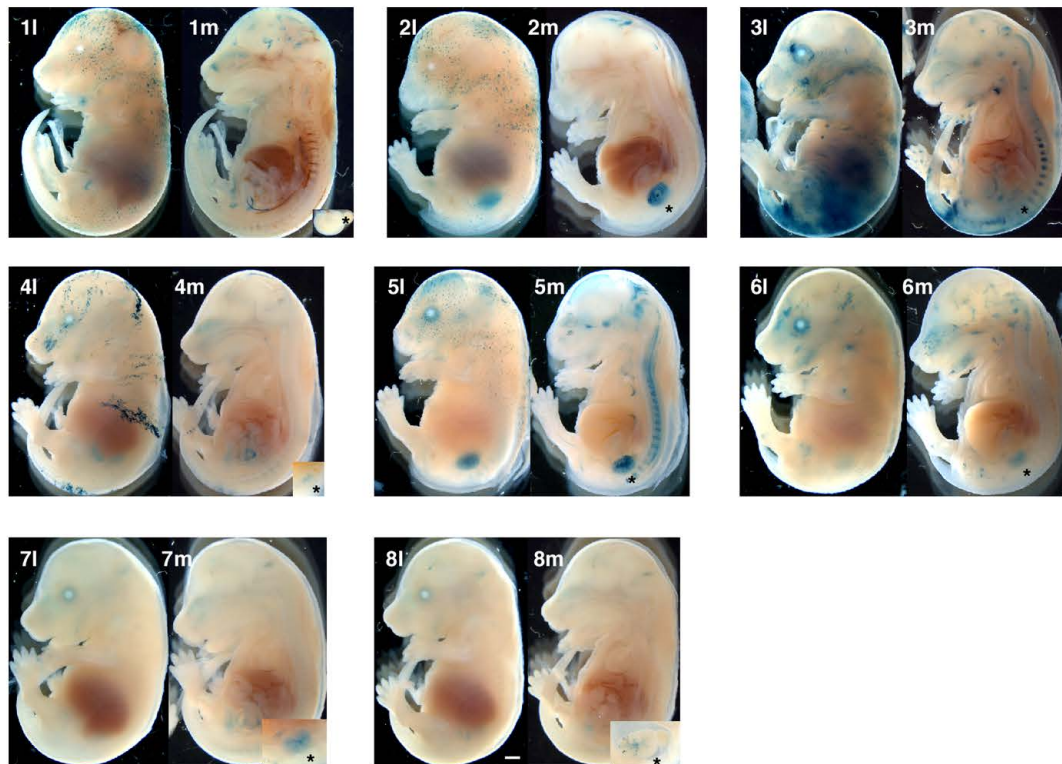
Supplementary Figure 2. H2b transgenic embryos. Thirteen transgenic embryos produced by pronuclear injection of the 1.5 kb H2b plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to show both external lateral (l) and internal medial (m) expression patterns. The kidney ($n = 12$) was the only consistent site of expression. The asterisk denotes the position of the kidney in the internal images. Scale bar, 1 mm.



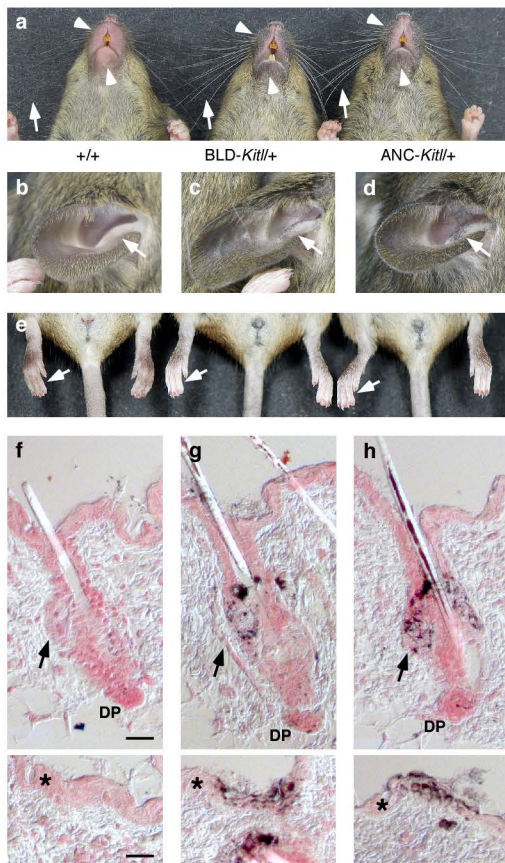
Supplementary Figure 3. HFE transgenic embryos. Eleven transgenic embryos produced by pronuclear injection of the 1.9 kb HFE clone are pictured. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to show both external lateral (l) and internal medial (m) expression patterns. Hair/skin expression was visible in 8 of the 11 embryos. The asterisk denotes the position of the kidney in the internal images. Scale bar, 1 mm.



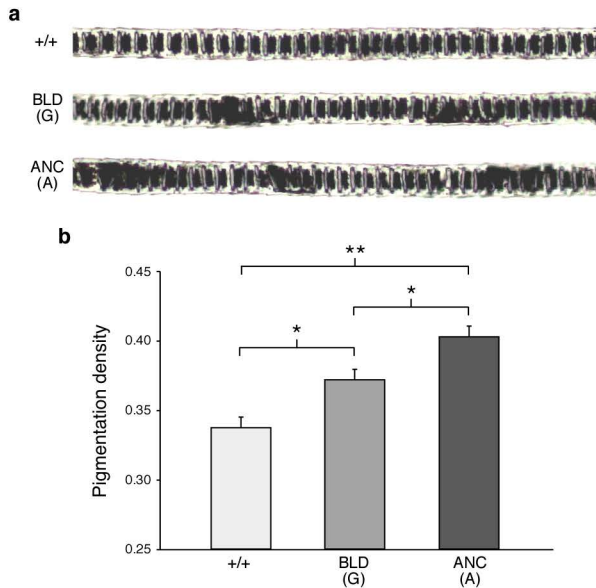
Supplementary Figure 4. H2-BLD transgenic embryos. Nine transgenic embryos produced by pronuclear injection of the 6.7 kb H2-BLD plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to show both external lateral (l) and internal medial (m) expression patterns. Hair/skin ($n = 7$) and kidney ($n = 7$) were consistent sites of expression. No clear difference in expression compared to the complete set of H2 (H2-ANC) transgenic embryos was evident (see Supplementary Fig. 1). The asterisk denotes the position of the kidney in the internal images. Scale bar, 1 mm.



Supplementary Figure 5. H2-DEL transgenic embryos. Eight transgenic embryos produced by pronuclear injection of the 6.7 kb H2-DEL plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to show both external lateral (l) and internal medial (m) expression patterns. Seven of the embryos show hair/skin expression. However the strength of this staining appeared reduced compared to H2-ANC and H2-BLD embryos, particularly in embryos that showed comparably strong kidney expression (such as embryos 2 and 5). The asterisk denotes the position of the kidney in the internal images. Scale bar, 1 mm.



Supplementary Figure 6. Additional pigmentation phenotypes seen in hair enhancer-*Kitl* mice. BLD-*Kitl* $+/+$ and ANC-*Kitl* $+/+$ heterozygotes exhibit several altered pigmentation patterns compared to wild-type (FVB/C57BL/6J F_1 hybrid) littermates. At 2 months, ectopic pigmentation is seen on the muzzles (arrowheads in **a**) and the epithelium of the antitragus and ear canal (arrows in **b-d**). In contrast, BLD-*Kitl* $+/+$ and ANC-*Kitl* $+/+$ heterozygotes show reduced pigmentation in the whiskers (arrows in **a**), and the hair on the digits (arrows in **e**), perhaps because of competitive interactions between body sites for melanocyte colonization and development⁴⁸ or premature differentiation of migrating melanocytes⁴⁹. (**f-h**) Cross-sections (6 μ m) through dorsal skin from 2-month-old (**f**) wild-type, (**g**) BLD-*Kitl* $+/+$, and (**h**) ANC-*Kitl* $+/+$ heterozygotes counterstained with nuclear fast red. Elevated *Kitl* expression controlled by both the BLD and ANC hair enhancers leads to ectopic pigmentation of the bulge region of hair follicles (arrows) and the basal epidermis (asterisks). DP, dermal papilla. Scale bars, 30 μ m.



Supplementary Figure 7. Analysis of pigment levels in zigzag hairs from site-specific transgenic mice. (a) Photographs of zigzag hairs from wild-type (+/+; FVB/C57BL/6J F₁ hybrid), BLD (BLD-*Kitl*/+) line 2, and ANC (ANC-*Kitl*/+) line 2 heterozygotes at P21. Fifteen hairs per mouse were analyzed to determine the fraction of pigmented pixels per hair shaft. (b) Mean pigmentation density in different genotypes. Both BLD-*Kitl*/+ and ANC-*Kitl*/+ heterozygotes exhibit significantly higher levels of pigmentation than wild-type controls. Notably, the amount of pigment in BLD-*Kitl*/+ heterozygotes is also significantly less than is found in ANC-*Kitl*/+ heterozygotes ($P = 0.0278$). Error bars indicate s.e.m. * $P < 0.05$, ** $P < 5 \times 10^{-3}$, unpaired t test.

Supplementary Table 1. LEF1 binding results from the Universal PBM Resource for Oligonucleotide Binding Evaluation (UniPROBE).

Sequence	Experimental Oligonucleotide	Z-score Exp1	Z-score Exp2	P value (combined)
LEF consensus	CATCAAAG	18.59	5.66	0.0000
Ancestral	C <u>A</u> CTAAAG	3.04	1.18	0.0024
Blond variant	C <u>G</u> CTAAAG	- 0.48	- 0.41	0.7283

In vitro binding of LEF1 protein to 8-mer oligonucleotides was measured using protein-binding microarrays as previously described³⁸. Z-scores for the indicated LEF1-oligonucleotide interactions are shown for two independent experiments, along with the *P* value for the combined results. Oligonucleotides with the ancestral A sequence at the position of the rs12821256 polymorphism (underlined) show significant binding interaction with LEF1 protein (*P* = 0.0024). In contrast, oligonucleotides with the blond-associated G variant at this position do not show significant binding. All data are from the UniPROBE database³⁹, and are organized here for their relevance to the human rs12821256 polymorphism.

Supplementary Table 2. Primers

Primers	Sequence 5'-3'	Assay/Clone	Size
Kg1514 Kg1521	GGATTGCGGCCGCTACTCCTTCTTATTGCTCTCTCTGTGG CAAAAAGAGTCCAGGACCAGACGGA	H1 partial	4.7 kb
Kg1025 Kg1026	GGATTGCGGCCGCTAATAGGGAACATAGAACACATGGAAG GGATTGCGGCCGCTATGATTGCTCATTGACACTGTGGTCG	H2	6.2 kb
Kg1516 Kg1517	GGATTGCGGCCGCTAGAAACGACCACAGTGTCAATGAGCA GGATTGCGGCCGCTATTAATGGTGCCAGCATCCGTCAGT	H3	3.1 kb
Kg1116 Kg1117	GGATTGCGGCCGCTATGCTTATATCCAGTTAAGGCTTTGGC GGATTGCGGCCGCTAAGGTACCTTTGCTTCTCGTGGGTCA	HFE-lacZ	1.9 kb
Kg1119 Kg1120	GGATTGCGGCCGCTAAGGCAAAGGACAACTCCCTGGAGA GGATTGCGGCCGCTAATCCAGAGCAGTATACCTCAAGGTG	H2b	1.5 kb
Kg1025 Kg1146	see above TAGGGTTTTTGGCGTAGTAACATGCCCTTGCT	H2del1	2.3 kb
Kg1147 Kg1026	TTACTACGGCAAAAACCCTAAACACAGAGCT see above	H2del2	4.4 kb
Kg1143 Kg1144	AGGATTACTCGAGTATGCTTATATCCAGTTAAGGCTTTGGC AGGATTACTCGAGTAAGGTACCTTTGCTTCTCGTGGGTCA	HFE-luciferase	1.9 kb
Kg1143 Kg1146	see above see above	HFEdel1	1.5 kb
Kg1147 Kg1144	see above see above	HFEdel2	384 bp
Kg1155 Kg1156	GTACCCTCGAGCGACACTAAAGGAGGTAACACTAAAGGAGGTAACACTAAAGGAGCGCGACACTAAAGG TAGATCTCGAGAATCCTTTAGTGTTACCTCCTTTAGTGTTACCTCCTTTAGTGTTACCTCCTTTAGTGT	7x ANC	112 bp
Kg1157 Kg1158	GTACCCTCGAGCGACGCTAAAGGAGGTAACGCTAAAGGAGGTAACGCTAAAGGAGCGCGACGCTAAAGG TAGATCTCGAGAATCCTTTAGCGTTACCTCCTTTAGCGTTACCTCCTTTAGCGTTACCTCCTTTAGCGT	7x BLD	112 bp
Kg1133 Kg1134	TAGGATCGAGCTCTAGACGATGTAGGTCACGGTCTCGAAG TAGGATCGAGCTCTAACATGCCCGCCGTGACCGTCGAGAA	attB	278 bp
Kg1135 Kg1136	CACTATAGGGCGAATTGGAGCTCCA TCTTCTTCATGGCGCCGCGCTCTGCTTCTGGA	Hspa1b	941 bp
Kg1137 Kg1138	GCGCGGCGCCATGAAGAAGACACAACTTGA CCGGTTATTATTACACCTCTTGAAATTCTCTC	Kitl	822 bp
Kg1139 Kg1140	AGAGGTGTAATAATAACCGGGCAGGGGGGATCTAA CACACAGGAAACAGCTATGACCATG	SV40pA	432 bp
Kg1118 Kg1117	GGATTGCGGCCGCTATGGGTAGGAGTGAGGAAAATGGCTA see above	HE-Kitl	864 bp
Kg1576	GCCATTTTCCTCACTCCTACCC	<i>Kitl</i> SSI genotyping	
Kg1580	GACACAAACTTGGATTATCACTTGCA	<i>Kitl</i> genotyping/ RT-QPCR	
Kg1581	GTTGAGGGTTATCATATAGTCATTTGG	<i>Kitl</i> genotyping/ RT-QPCR	
Kg1588	ACAGCTTCTTGCAGCTCCTT	<i>Actb</i> RT-QPCR	
Kg1589	ATAGGAGTCCTTCTGACCCAT	<i>Actb</i> RT-QPCR	

Additional References

48. Wehrle-Haller, B., Morrison-Graham, K. & Weston, J.A. Ectopic c-kit expression affects the fate of melanocyte precursors in Patch mutant embryos. *Dev. Biol.* **177**, 463-74 (1996).
49. Kunisada, T. *et al.* Transgene expression of steel factor in the basal layer of epidermis promotes survival, proliferation, differentiation and migration of melanocyte precursors. *Development* **125**, 2915-2923 (1998).