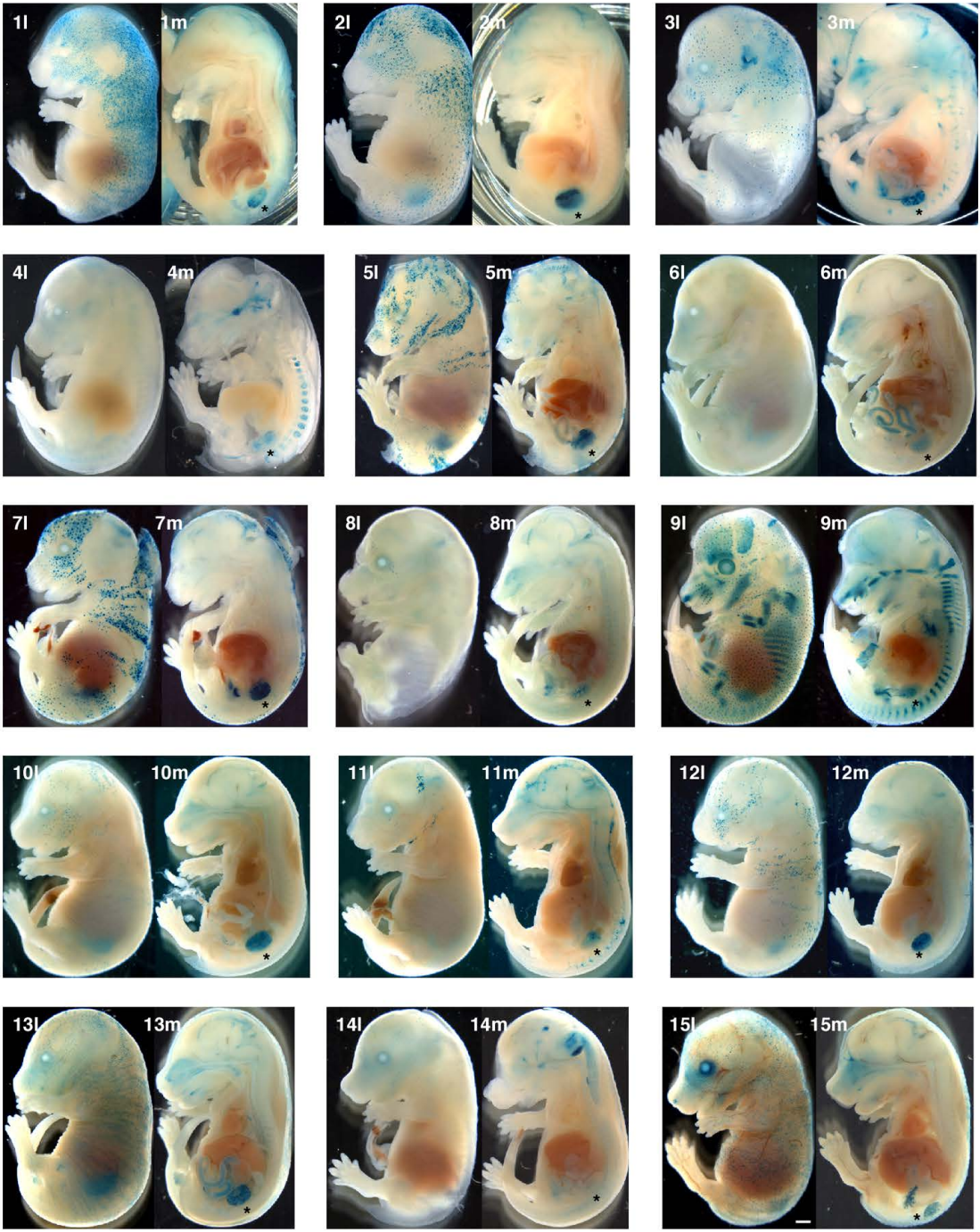
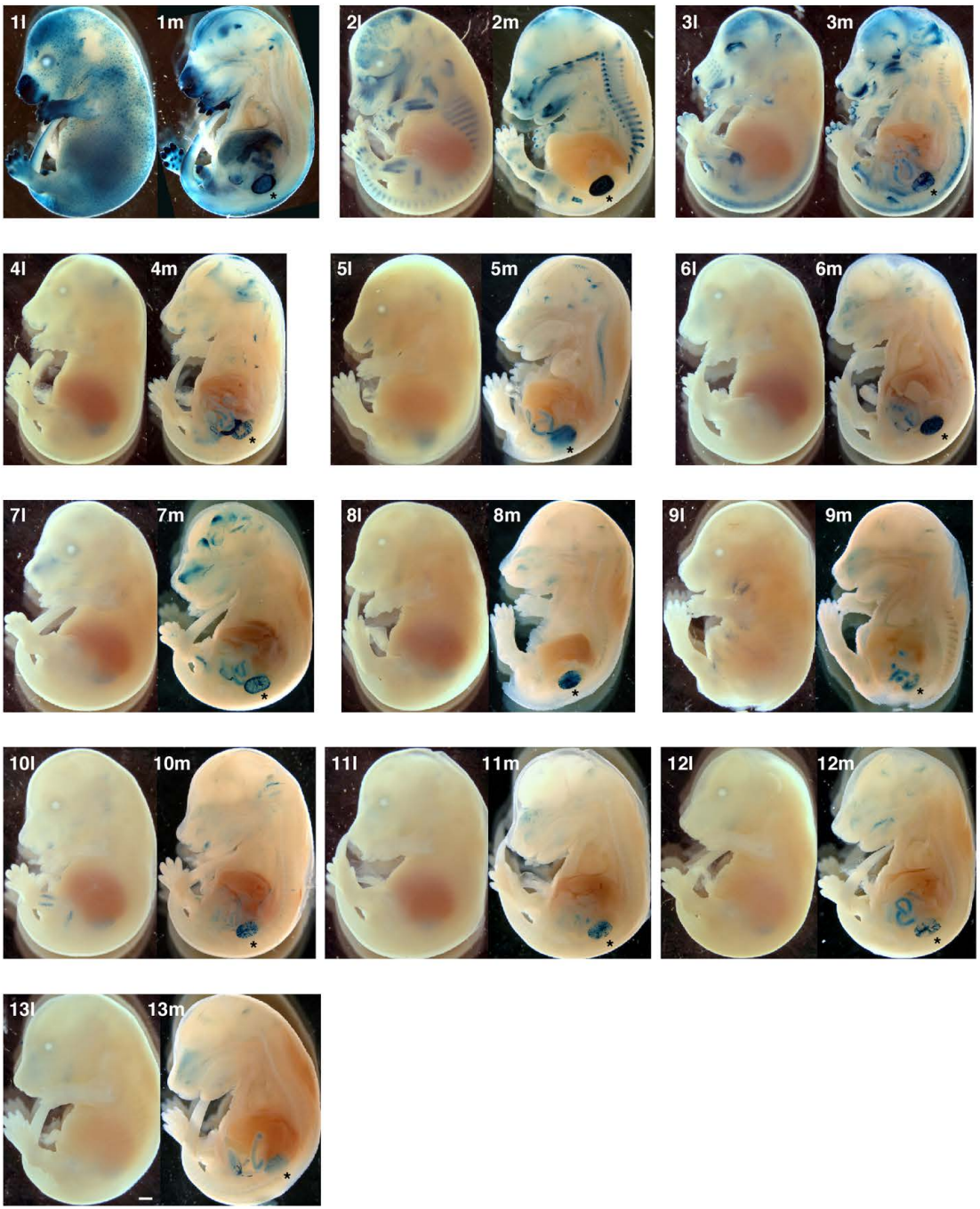
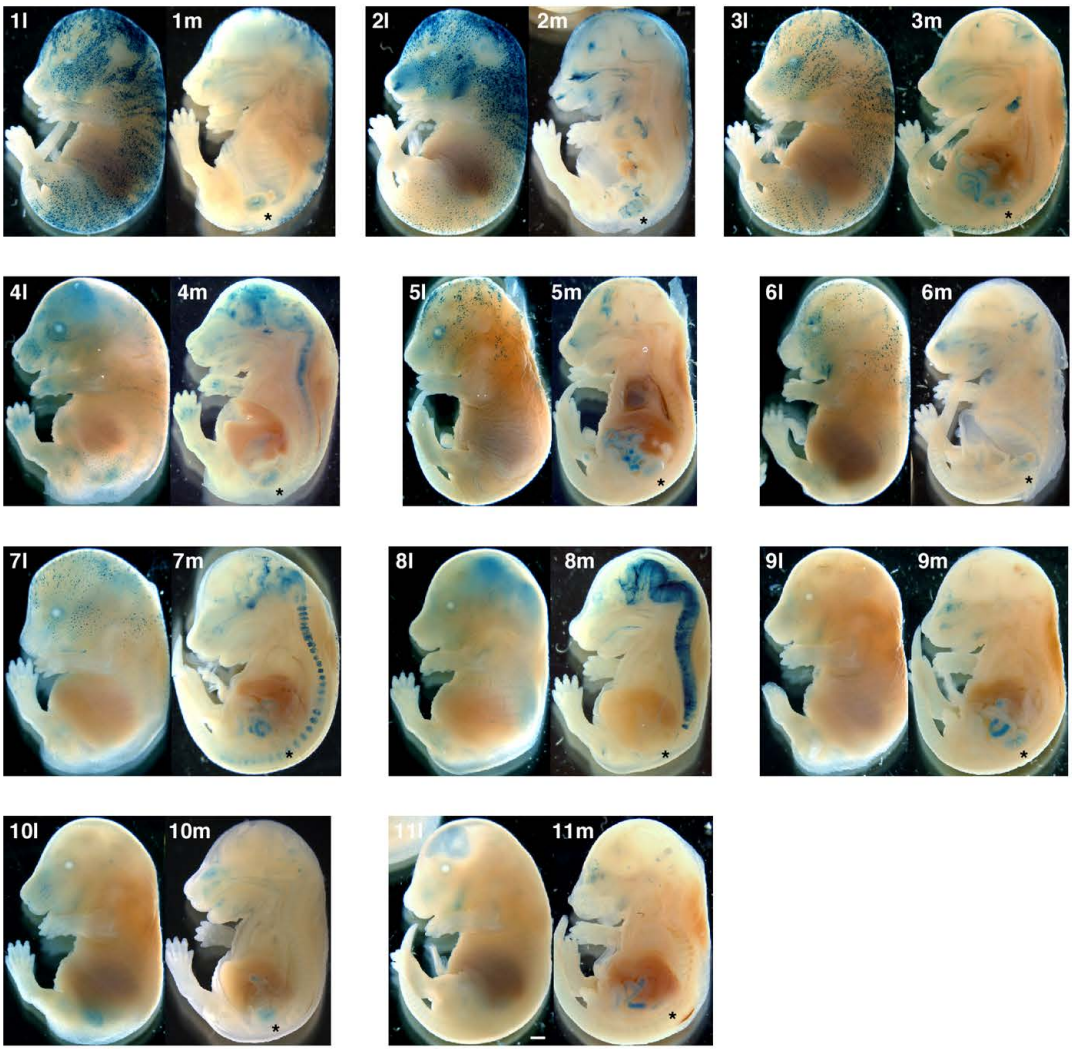


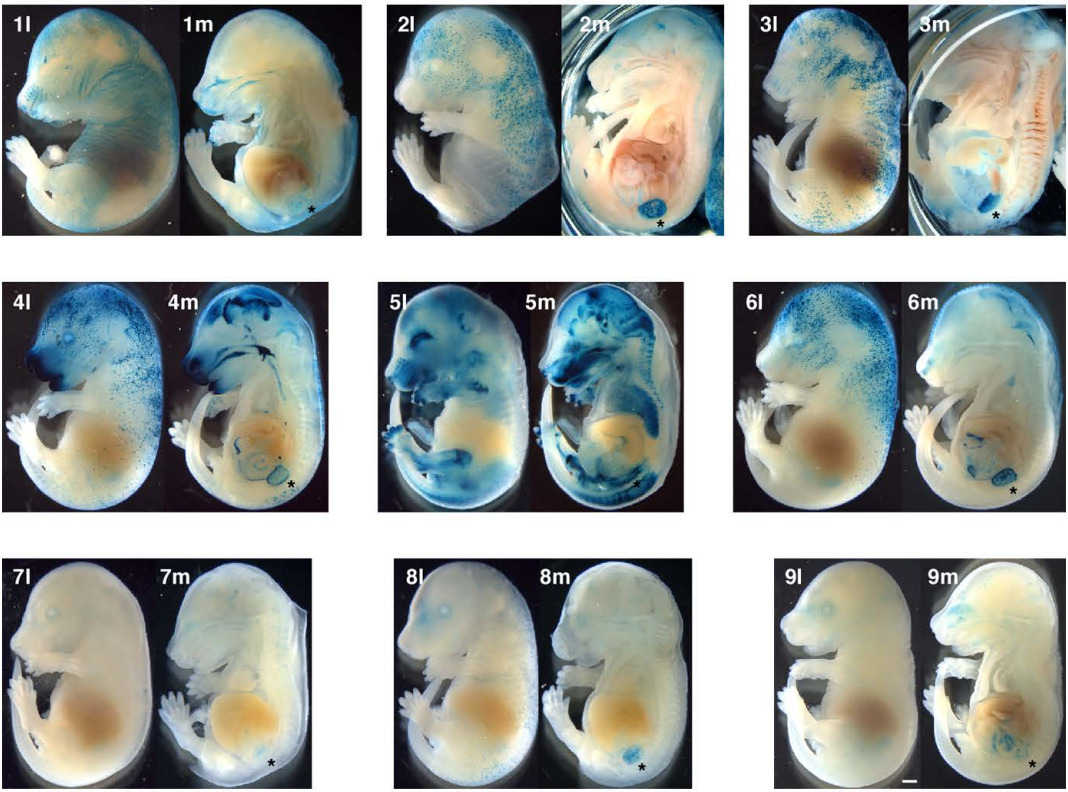
Supplementary Figure 1. H2 transgenic embryos. Fifteen transgenic embryos produced by pronuclear injection of the 6.7 kb H2 plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to 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.



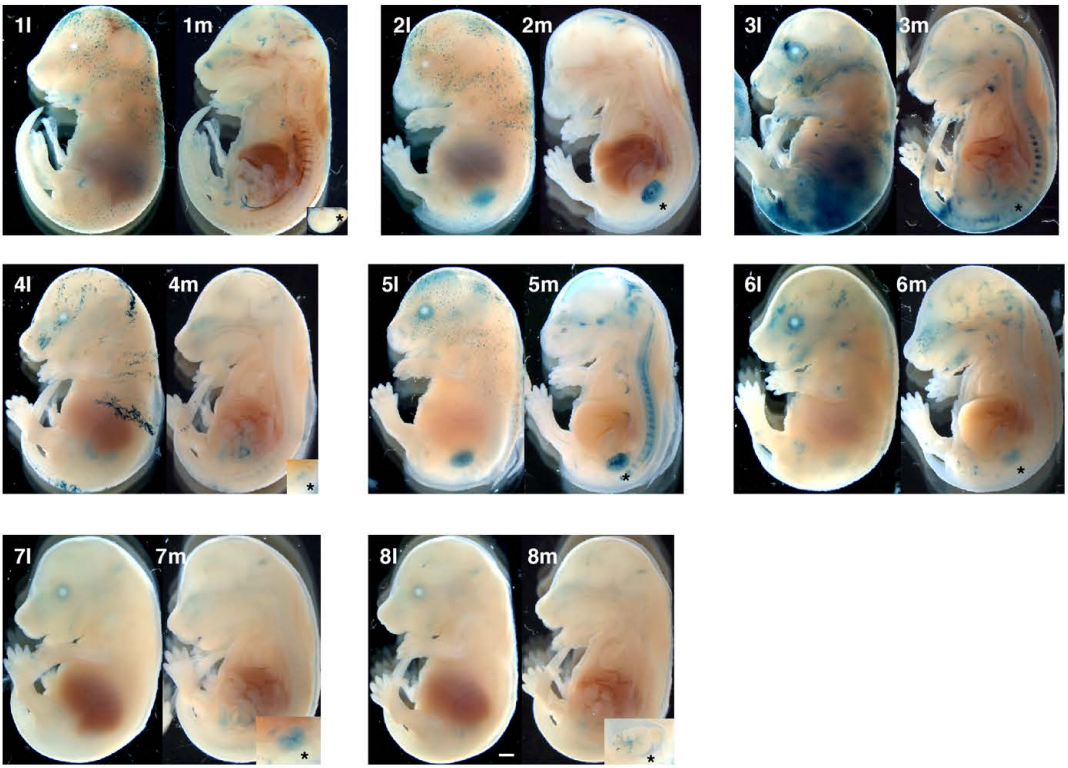
Supplementary Figure 2. H2b transgenic embryos. Thirteen transgenic embryos produced by pronuclear injection of the 1.5 kb H2b plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging 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.



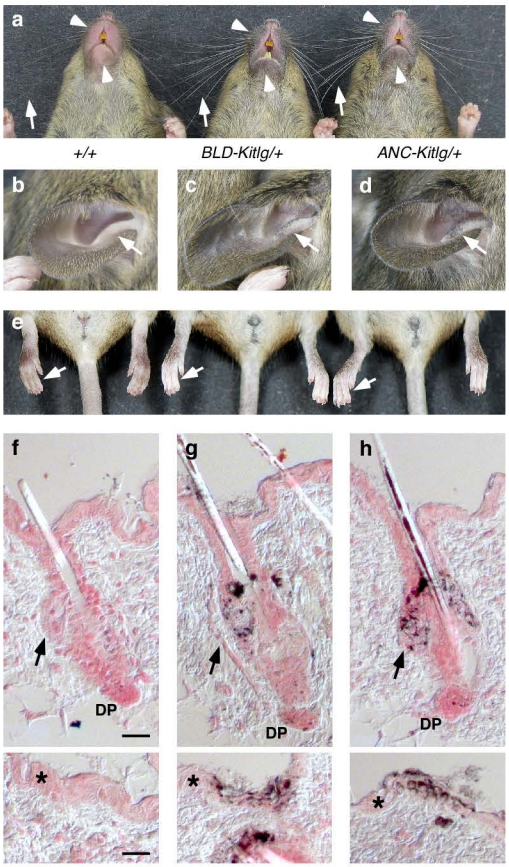
Supplementary Figure 3. HFE transgenic embryos. Eleven transgenic embryos produced by pronuclear injection of the 1.9 kb HFE clone are pictured. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging to 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.



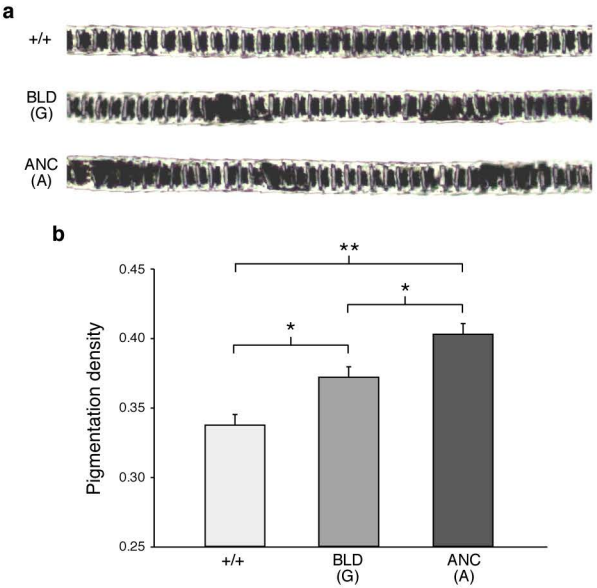
Supplementary Figure 4. H2-BLD transgenic embryos. Nine transgenic embryos produced by pronuclear injection of the 6.7 kb H2-BLD plasmid are shown. Each embryo represents an independent genomic integration event. Embryos were collected at E16.5, stained for *lacZ* activity, and bisected before imaging 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 animals 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-Kitlg* mice. *BLD-Kitlg/+* and *ANC-Kitlg/+* heterozygotes exhibit several altered pigmentation patterns compared to wild type (FVB/C57BL/6J F1 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-Kitlg/+* and *ANC-Kitlg/+* 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 development[48] or premature differentiation of migrating melanocytes[49]. (**f-h**) 6 µm cross-sections through dorsal skin from 2-month-old (**f**) wild type, (**g**) *BLD-Kitlg/+*, and (**h**) *ANC-Kitlg/+* heterozygotes counterstained with nuclear fast red. Elevated Kitlg 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 µm.



Supplementary Figure 7. Analysis of pigment levels in zigzag hairs from site-specific transgenic mice. (**a**) Photographs of zigzag hairs from wild type (*+/+*; FVB/C57Bl6/J F1 hybrid), BLD (*BLD-Kitlg/+*) line 2, and ANC (*ANC-Kitlg/+*) line 2 heterozygotes at P21. Fifteen hairs per animal were analyzed to determine the fraction of pigmented pixels per hair shaft. (**b**) Mean pigmentation density in different genotypes. Both *BLD-Kitlg/+* and *ANC-Kitlg/+* heterozygotes exhibit significantly higher levels of pigmentation than wild type controls. Notably, the amount of pigment in *BLD-Kitlg/+* heterozygotes is also significantly less than is found in *ANC-Kitlg/+* heterozgyotes (P=0.0278). Error bars indicate s.e.m. Unpaired t test values; * $P<0.05$, ** $P<5x10^{-3}$.

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**A**CTAAAG	3.04	1.18	0.0024
Blond variant	C**G**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 described[38]. 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 database[39], and are organized here for their relevance to the human rs12821256 polymorphism.

Supplementary Table 2. Primers

Primers	Sequence 5'-3'	Assay/Clone	Size
Kg1514	GGATTGCGGCCGCTACTCCTTCTTATTGCTCTCTCTGTGG	H1 partial	4.7 kb
Kg1521	CAAAAAGAGTCCAGGACCAGACGGA		
Kg1025	GGATTGCGGCCGCTAATAGGGAACATAGAACACATGGAAG	H2	6.2 kb
Kg1026	GGATTGCGGCCGCTATGATTGCTCATTGACACTGTGGTCG		
Kg1516	GGATTGCGGCCGCTAGAAACGACCACAGTGTCAATGAGCA	H3	3.1 kb
Kg1517	GGATTGCGGCCGCTATTAAATGGTGCCAGCATCCGTCAGT		
Kg1116	GGATTGCGGCCGCTATGCTTATATCCAGTTAAGGCTTTGGC	HFE-lacZ	1.9 kb
Kg1117	GGATTGCGGCCGCTAAGGTACCTTTGCTTCTCGTGGGTCA		
Kg1119	GGATTGCGGCCGCTAAGGCAAAGGACAAACTCCCTGGAGA	H2b	1.5 kb
Kg1120	GGATTGCGGCCGCTAATCCAGAGCAGTATACCTCAAGGTG		
Kg1025	see above	H2del1	2.3 kb
Kg1146	TAGGGTTTTTTGCCGTAGTAACATGCCCTTGGCT		
Kg1147	TTACTACGGCAAAAAACCCTAAACACAGAGCT	H2del2	4.4 kb
Kg1026	see above		
Kg1143	AGGATTACTCGAGTATGCTTATATCCAGTTAAGGCTTTGGC	HFE-luciferase	1.9 kb
Kg1144	AGGATTACTCGAGTAAGGTACCTTTGCTTCTCGTGGGTCA		
Kg1143	see above	HFEdel1	1.5 kb
Kg1146	see above		
Kg1147	see above	HFEdel2	384 bp
Kg1144	see above		
Kg1155	GTACCCTCGAGCGACACTAAAGGAGGTAACACTAAAGGAGGTAACACTAAAGGAGCGCGACACTAAAGG	7X ANC	112 bp
Kg1156	TAGATCTCGAGAATCCTTTAGTGTTACCTCCTTTAGTGTTACCTCCTTTAGTGTTACCTCCTTTAGTGT		
Kg1157	GTACCCTCGAGCGACGCTAAAGGAGGTAACGCTAAAGGAGGTAACGCTAAAGGAGCGCGACGCTAAAGG	7X BLD	112 bp
Kg1158	TAGATCTCGAGAATCCTTTAGCGTTACCTCCTTTAGCGTTACCTCCTTTAGCGTTACCTCCTTTAGCGT		
Kg1133	TAGGATCGAGCTCTAGACGATGTAGGTCACGGTCTCGAAG	attB	278 bp
Kg1134	TAGGATCGAGCTCTAACATGCCCGCCGTGACCGTCGAGAA		
Kg1135	CACTATAGGGCGAATTGGAGCTCCA	hsp68	941 bp
Kg1136	TCTTCTTCATGGCGCCGCGCTCTGCTTCTGGA		
Kg1137	GCGCGGCGCCATGAAGAAGACACAAACTTGGA	Kitlg	822 bp
Kg1138	CCGGTTATTATTACACCTCTTGAAATTCTCTC		
Kg1139	AGAGGTGTAATAATAACCGGGCAGGGGGGATCTAA	SV40pA	432 bp
Kg1140	CACACAGGAAACAGCTATGACCATG		
Kg1118	GGATTGCGGCCGCTATGGGTAGGAGTGAGGAAAATGGCTA	HE-Kitlg	864 bp
Kg1117	see above		
Kg1576	GCCATTTTCCTCACTCCTACCC	Kitlg SSI genotyping	
Kg1580	GACACAAACTTGGATTATCACTTGCA	Kitlg genotyping/ RT-QPCR	
Kg1581	GTTGAGGGTTATCATATAGTCATTTGG	Kitlg genotyping/ RT-QPCR	
Kg1588	ACAGCTTCTTTGCAGCTCCTT	Actb RT-QPCR	
Kg1589	ATAGGAGTCCTTCTGACCCAT	Actb 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).