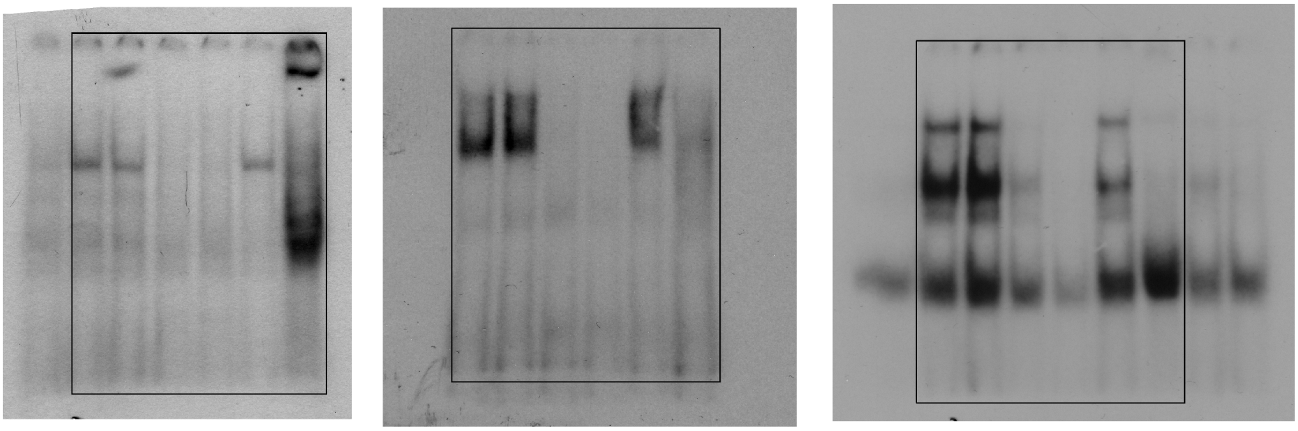
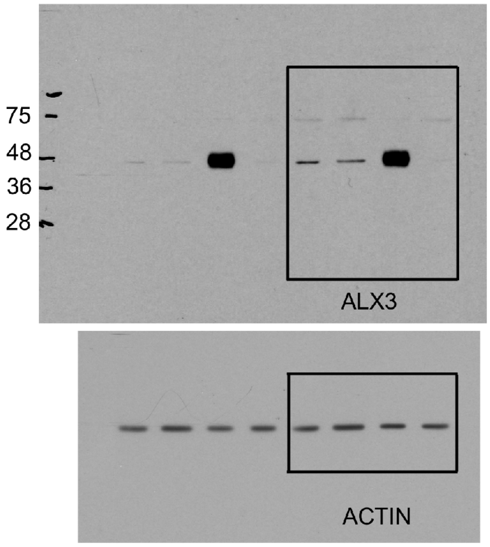
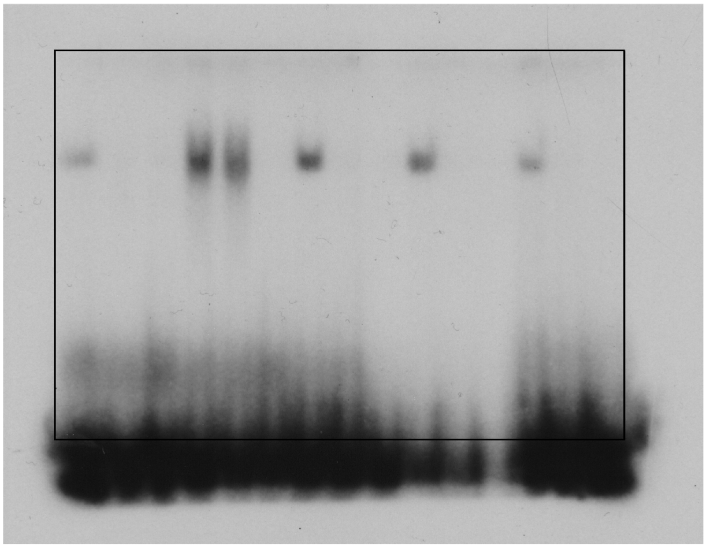


Extended Data Figure 10a



Extended Data Figure 6b



Extended Data Figure 10b

Supplementary Table 1a

Gene symbol	Mean count across all samples	Log2 fold-change Light/Dark	P-value	Q-value (FDR)
ALX3	56	2.75	7.82E-22	1.16E-17
ASIP	131	1.9	6.04E-14	3.00E-10
DCT	1917	-0.15	2.58E-02	5.35E-01
EDN3	643	-0.49	1.26E-08	2.14E-05
GPR143	360	-0.67	3.11E-08	4.21E-05
KIT	1682	-0.06	2.85E-01	9.87E-01
MC1R	605	-0.45	2.19E-07	1.72E-04
MITF	1357	-0.12	2.84E-02	5.60E-01
MLANA	435	-0.31	1.14E-02	3.78E-01
OCA2	398	-0.98	4.52E-08	4.80E-05
POMC	19	-0.16	4.55E-01	1.00E+00
SLC7A11	418	0.02	8.28E-01	1.00E+00
TBX3	294	-0.01	9.45E-01	1.00E+00
TRPM1	1012	-0.52	7.01E-06	2.98E-03
TYR	1968	-0.77	3.07E-10	7.61E-07
TYRP1	4871	-0.96	1.56E-07	1.29E-04

Supplementary Table 1b

Gene symbol	Mean count across all samples	Log2 fold-change Light/Flank	P-value	Q-value (FDR)
ALX3	56	2.11	2.47E-14	4.09E-11
ASIP	131	0.32	1.88E-01	5.22E-01
DCT	1917	-0.11	9.74E-02	3.67E-01
EDN3	643	0.01	8.71E-01	9.56E-01
GPR143	360	0.21	8.89E-02	3.50E-01
KIT	1682	0.02	7.32E-01	9.07E-01
MC1R	605	0.21	1.39E-02	1.11E-01
MITF	1357	0.17	1.61E-03	2.41E-02
MLANA	435	0.2	9.79E-02	3.68E-01
OCA2	398	0.33	6.75E-02	2.99E-01
POMC	19	-0.16	4.26E-01	7.53E-01
SLC7A11	418	0.3	4.23E-03	4.86E-02
TBX3	294	-0.32	2.56E-03	3.33E-02
TRPM1	1012	0.3	1.04E-02	9.06E-02
TYR	1968	0.18	1.44E-01	4.52E-01
TYRP1	4871	0.16	3.85E-01	7.17E-01

Supplementary Table 1c

Gene symbol	Mean count across all samples	Log2 fold-change Dark/Flank	P-value	Q-value (FDR)
ALX3	56	0.64	3.21E-02	2.27E-01
ASIP	131	1.58	5.31E-10	2.35E-07
DCT	1917	-0.04	5.66E-01	8.60E-01
EDN3	643	-0.5	4.79E-09	1.57E-06
GPR143	360	-0.87	4.94E-13	4.36E-10
KIT	1682	-0.08	1.58E-01	5.12E-01
MC1R	605	-0.66	2.42E-14	2.63E-11
MITF	1357	-0.29	9.32E-08	1.88E-05
MLANA	435	-0.51	2.92E-05	2.24E-03
OCA2	398	-1.31	3.15E-13	2.97E-10
POMC	19	0.01	9.66E-01	NA
SLC7A11	418	-0.27	8.37E-03	1.02E-01
TBX3	294	0.31	3.26E-03	5.82E-02
TRPM1	1012	-0.81	1.78E-12	1.26E-09
TYR	1968	-0.95	8.79E-15	1.13E-11
TYRP1	4871	-1.12	9.67E-10	3.80E-07

Supplementary Table 1. Detailed results of RNA-Seq analysis for pigmentation genes. a, Samples obtained from the light and dark stripes. **b,** Samples obtained from the light stripe and the flank. **c,** Samples obtained from the dark stripe and the flank. Four columns are included for each detected gene, displaying the average overall expression level (normalized gene counts), a $\log_2$ fold-change between samples from the compared regions, as well as the degree of statistical significance of the observed differences expressed as *P*-values and *Q*-values (adjusted for multiple testing using the Benjamini and Hochberg correction approach). Genes are sorted alphabetically. The data presented in this table was obtained from gene counts computed using eXpress software and RNA-seq read alignments against the de novo transcriptome assembly.

Supplementary Table 2. Oligo sequences. Sequences used for quantitative PCR (qPCR), ChIP-qPCR, and EMSA.

Species	Gene/Site	Experiment	Forward primer	Reverse primer
R. pumilio/M. musculus	*Alx3*	qPCR	GTTCCAGAACCGAAGAGCCA	GCTGAGGATGGCTGTCAGTT
R. pumilio/M. musculus	*Tyr*	qPCR	ACCATGACAAAGCCAAAACC	GGCAAATCCTTCCAGTGTGT
R. pumilio/M. musculus	*Tyrp1*	qPCR	CTGGGTTTCTCCTGATTGG	GCCCCAACTCTGTCTTTTC
R. pumilio/M. musculus	*Pmel*	qPCR	GCACCCAACTTGTTGTTCCT	AGAGATGCAAGGACCACAGC
R. pumilio/M. musculus	*Bactin*	qPCR	GCTACAGCTTCACCACCACA	TCTCCAGGGAGGAAGAGGAT
M. musculus	*Mitf*	qPCR	ACTTTCCCTTATCCCATCCAC	TGAGATCCAGAGTTGTCGTACA
T. striatus	*Alx3*	qPCR	AATGTGGCTGGCTTCATGGG	CCATCACGTGGTCCAGTTCA
T. striatus	*Asip*	qPCR	GTGGCACTGAACAAGAAATC	TTGCAGCTGTCGCGGGTGGC
T. striatus	*Edn3*	qPCR	ACAAGGACAAGGAGTGTGTC	TGGACAGTCCATAGGGCACA
T. striatus	*Bactin*	qPCR	CCACCATGTACCCAGGCATT	ACTCCTGCTTGCTGATCCAC
M. musculus	Sites 1, 2	EMSA	TGATTCATTAAAAAAGGGGGGCTCTGCCAGTGAATTAGTGTT	AAACACTAATTCACTGGCAGAGCCCCCCTTTTTTAATGAATC
M. musculus	Site 3	EMSA	TCTATTGTCCCATCATTATCGCGTCTG	CCAGACGCGATAATGATGGGACAATAG
M. musculus	Site 4	EMSA	GATTTTGACAGAATTAATGAGTTGCAAATA	TATTTGCAACTCATTAATTCTGTCAAAATC
M. musculus	Site 5	EMSA	GCAGCATTTATCCTAATTTCTAAATATAGA	TCTATATTTAGAAATTAGGATAAATGCTGC
M. musculus	Sites 6, 7	EMSA	ATGCTTCCTAATTTGAGGATTAGCTGAGAA	TTCTCAGCTAATCCTCAAATTAGGAAGCAT
M. musculus	Site 8	EMSA	CACACATGGCATCATTATTTGGCCATTAGG	CCTAATGGCCAAATAATGATGCCATGTGTG
M. musculus	Site 9	EMSA	AACACCATTGTCCATTAATACTACTGGAACGAAA	TTTCGTTCCAGTAGTATTAATGGACAATGGTGTT
M. musculus	Site 10	EMSA	GCATAACTAATTAGCTTAGGTTATTATAAG	GCTTATAATAACCTAAGCTAATTAGTTATG
M. musculus	*Mitf* Site 3	ChIP-qPCR	CTCCTGCCCTCATTTCGTGT	TCGCAGGCATTTTCAGTTTTGT
M. musculus	*Mitf* Site 5	ChIP-qPCR	CATCCCATTTCAACAGTAAGCAGC	AGTCCGTTTGCCATAAGCAGT
M. musculus	*Mitf* Site 10	ChIP-qPCR	CATGACGTCAAGCCAGCAG	CACCGCCGGAGACGTTATC
M. musculus	*Tyr*	ChIP-qPCR	AGTCATGTGCTTTGCAGAAGAT	CAGCCAAGAACATTTTCTCCTT

Supplementary Table 3. shRNA constructs. Clone ID, target sequences and hairpin sequences for the shRNA constructs used.

shRNA number	Clone ID	Target sequence	Hairpin sequence
1	TRCN0000081718	GCTAGTCTACAGAGATACTAT	CCGG-GCTAGTCTACAGAGATACTAT-CTCGAG-ATAGTATCTCTGTAGACTAGC-TTTTTG
2	TRCN0000081719	CCTGGCATCTATTCCATCCAT	CCGG-CCTCGTTTCACTCAGGATGAA-CTCGAG-TTCATCCTGAGTGAAACGAGG-TTTTTG
3	TRCN0000081720	CCTGGCATCTATTCCATCCAT	CCGG-CCTGGCATCTATTCCATCCAT-CTCGAG-ATGGATGGAATAGATGCCAGG-TTTTTG
4	TRCN0000081722	GCGTGAGCGTTATGGGAAGAT	CCGG-GCGTGAGCGTTATGGGAAGAT-CTCGAG-ATCTTCCCATAACGCTCACGC-TTTTTG
scrambled	Addgene clone 1864		