SUPPLEMENTAL INFORMATION

SUPPLEMENTAL MATERIALS AND METHODS

Mouse strains and genotyping

Genomic DNA was isolated from distal toe clips of altricial animals using the Quick Genotyping DNA Preparation Kit (Bioland Scientific, LLC) according to the manufacturer's instructions. Genomic DNA was sequenced to characterize the deletions of mice containing CRISPR/Cas9 generated deletions in *Mitf* at the Roy J. Carver Biotechnology Center (*https://biotech.illinois.edu/htdna*). Following the identification of 7bp deletion in exon 1A of *Mitf*, the effect on protein sequence was visualized in SnapGene Viewer (version 4.1.2) (from GSL Biotech; available at snapgene.com). After characterization of deletions, genotypes of progeny were determined using designed genotyping primers for *Mitf* mice. For the *Mitf-A* line, wildtype and homozygous knockouts are indistinguishable, so genotyping PCR reaction product was purified using the QIAquick PCR Purification Kit (Qiagen) and submitted for Sanger Sequencing (GENEWIZ) to determine whether the 7bp deletion was present. To identify the presence of *Crb1 rd8* mutation present in mutant mice generated from the C57BL/6NTac line, we used established genotyping primers (Table S5). C57BL/6J albino mice purchased from The Jackson Laboratories and C57BL/6N mice obtained from the KOMP repository (www.komp.org) were used as wildtype and *rd8* mutant controls, respectively, to verify the mixed C57BL/6NTac and C57BL/6J background. Allele frequencies for wildtype and *rd8* alleles were calculated based on genotyping of approximately 50 mice of each *Mitf* line.

Single cell isolation and sorting continued

Fat was scrapped off isolated back skin of $ROSA^{mTmG}$ mice using a scalpel, and then the skin was cut into approximately 2mm by 2mm pieces. All pieces of each skin were placed into a Gentlemacs C Tube (Miltenyi) digested for 1 hour in an enzyme cocktail containing 232U DNAse I (Sigma-Aldrich), 0.25mg/ml liberase (Sigma-Aldrich), 23.2mM HEPES, 2.32mM sodium pyruvate (Corning), 0.0025g hyaluronidase (Fisher Scientific), and 1mg/mL dispase:collagenase (Sigma-Aldrich) in 5mL RPMI 1640 (Corning). Dissociation was stopped with 10µL of 0.5M EDTA and 400µL FBS. The skin was fully dissociated using gentlemacs m_imptumor_01 protocol (Miltenyi Biotec). Each sample was filtered twice in fresh RPMI containing FBS.

RNA sequencing

Total RNA from GFP+ and tdTomato+ cells from mice 1-4 was monitored for quality control using the Agilent Bioanalyzer Nano RNA chip. Library construction was performed according to the Illumina TruSeq RNA v2 protocol. The input quantity for total RNA was 1µg, and mRNA was enriched using oligo

dT magnetic beads. The enriched mRNA was chemically fragmented for four minutes. First strand cDNA synthesis was performed using random primers, dNTPs, and reverse transcriptase to make cDNA. After second strand synthesis the cDNA was cleaned using AMPure XP beads, the cDNA was end repaired, and then the 3' ends were adenylated. Illumina barcoded adapters were ligated on the ends, and the adapter ligated fragments were enriched through nine cycles of PCR. The resulting libraries were validated by qPCR and sized by Agilent Bioanalyzer DNA high sensitivity chip. The concentrations for the libraries were normalized and then multiplexed together. The concentration for clustering on the flowcell was 12.5pM. The multiplexed libraries were sequenced on three lanes using paired-end 100 cycles chemistry for the HiSeq 2500. The version of HiSeq control software used was HCS 2.2.38 with real time analysis software, RTA 1.18.61. Paired-end sequencing reads were trimmed of adapter sequences, analyzed for quality using the Fastqc program (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and aligned to the mouse reference genome version mm10 using the Tophat alignment software (version 2.0.12) (Trapnell, Pachter, & Salzberg, 2009). Fragments and exons were quantified using the Cufflinks program (version 2.2.1) (Trapnell et al., 2013). DEseq2 (version 1.14.1) (Love, Huber, & Anders, 2014) was utilized to distinquish differentially expressed genes from GFP+ and tdTomato+ populations in R (3.3.2).

Cell lines and cell culture

Human MNT-1 melanoma cells, a gift from M. Marks (University of Pennsylvania), were cultured in high-glucose DMEM (containing sodium pyruvate and L-glutamine) (Caisson) supplemented with 15% fetal bovine serum (CellGro), MEM vitamin solution (Invitrogen), antibiotic-antimycotic (Invitrogen), and 10% AIM-V medium (Invitrogen). Human deeply-pigmented neonatal epidermal melanocytes (Invitrogen) were cultured in Medium 254 (Invitrogen) supplemented with phorbol 12-myristate 13-acetate-free Human Melanocyte growth supplement-2 (Invitrogen). As these melanocyte strains were purchased from commercial entities, no IRB approval was required prior to their use.

Reverse-transcriptase quantitative PCR (RT-qPCR)

Primers were designed to target the unique amino-terminus of human *MITF-A* and *MITF-M* using Primer3 (Koressaar & Remm, 2007; Untergasser et al., 2012). Primers for the unique first exons of *Mitf* isoforms were previously described (Bharti, Liu, Csermely, Bertuzzi, & Arnheiter, 2008) with additional primers designed utilizing Primer3. All primers used are listed in Table S4. Power SYBR Green PCR master mix (Life Technologies) was used to amplify pure cDNA. All reactions were completed in technical and biological triplicate (n=3). A 7900HT Fast Real-Time PCR system (Applied Biosystems) and SDS 2.4 (Applied Biosystems) were used to determine Ct values for each sample. Values for human genes were

normalized to either *β-actin* or *GAPDH* and values from mouse tissue were normalized to *Hprt* using the relative quantification mathematical model (Pfaffl) as previously described (Paterson, Ho, Kapadia, & Ganesan, 2013). A two-tailed Student's t-test and two-way ANOVA were employed to determine statistical significance with R statistical Software (version 3.3.2).

To confirm the mutation present in *Mitf-A* transcripts from *Mitf-A* mutant mice, cDNA from wild-type and *Mitf-A* knockout mice was amplified using a sequencing forward primer that was upstream of the deletion and the common Exon 1 Reverse qPCR primer. PCR reaction product was purified using the QIAquick PCR Purification Kit (Qiagen) and submitted for Sanger Sequencing (GENEWIZ) to determine whether the 7bp deletion was present. The sequences were aligned to the mouse *Mitf-A* transcript (NM_001113198.1) in SnapGene (version 4.2.6) with primers and amino acid sequence labeled.

Nanostring nCounter analysis on whole-mouse skin

Mice were shaved and depilated at P50 to stimulate the 3rd Anagen. Whole mouse skin was harvested from euthanized mice at P60 and immediately stabilized overnight in RNAlater (Invitrogen) at 4°C. Skin samples were homogenized using the Precellys24 high-throughput tissue homogenizer (Precellys) in hard tissue homogenizing reinforced tubes that contain 2.8mm ceramic beads (Bertin Corporation). After homogenization, RNA was extracted from each sample using the RNeasy Fibrous Tissue Mini Kit according to the manufacturer's instructions (Qiagen). RNA was normalized to a concentration of 20ng/μL and 5μL of normalized sample was then added to a 20μL aliquot of reporter codeset master mice. The samples were then hybridized at 65°C for 16-18 hours, and then transferred to the prep station. The prep station was run on the high sensitivity setting. Upon completion of the prep station step, the cartridge was loaded onto the nCounter and scanned using the "max" setting which attempts to capture 1155 fields of view (FOV). Resultant data was analyzed using the nSolver analysis software (Nanostring Technologies).

Western blots and immunoprecipitation

Both eyes from adult mice were incubated in LysisT buffer (250mM NaCl; 50mM Tris, pH 7.5; 0.125% Na deoxycholate; 0.375% Triton X-100; 0.15% NP-40; 4mM EDTA; 10μM Aprotinin; 50μM Leupeptin; 1mM PMSF) with Halt Protease and Phosphatase Inhibitor (Thermo Scientific) for 10 minutes. The samples were homogenized using the Precellys24 high-throughput tissue homogenizer as described above. Next, the samples were incubated on ice for 30 minutes prior to centrifugation to collect protein lysate. Lysates were subjected to SDS-PAGE on 8% Tris-Glycine gels and transferred onto Immobilon-P membranes (EMD Millipore). Membranes were blocked in 5% non-fat milk with 1X TBS and 1% Tween-

20, and probed with rabbit polyclonal GAPDH, 5174, lot# 6; rabbit monoclonal MITF, 12950, lot# 2 (Cell Signaling Technology). Protein levels were assessed using densitometry analysis (ImageJ, NIH).

Immunofluorescence Staining

Skin from mice were collected at P56 and processed following established protocols (Harris et al., 2018). Dissected skin was cut into 1cm x 1cm square and fixed in 2% formaldehyde in 1X PBS for 30 minutes on ice. Fixed tissue was washed overnight in PBS at 4°C, followed by an overnight wash in 10% sucrose in PBS at 4°C. Skin was embedded in OCT (Fisher Scientific) and stored at -80°C. Ten-micron sections of skin were cut and dried overnight at room temp. Slides were blocked in 1% BSA (Fisher Scientific) in 1X PBS with 0.1% Tween-20 (Fisher Scientific) and probed with rat monoclonal CD117, CL8936AP, lot# 103628G (Cedarlane) and chicken anti-rat, Alexa488 (Life Technologies). Slides were imaged using Nikon Eclipse Ti fluorescent microscope. The number of CD117 positive hair follicles was counted in two distinct (non-sequential) slides and averaged for each mouse. A one-way ANOVA was used to calculate statistical significance with R statistical software (version 3.5.1). Images were post processed using Adobe Photoshop.

Eyes collected at P56 were fixed and cut in half prior to embedding, each half of the eye was embedded separately. Sectioning and staining were completed as described above.

Kidney Characterization

Kidneys were collected from 4-month-old mice of both genders (3 males, 3 females for each genotype). The ratio of kidney to body mass was calculated from the combined weight of both kidneys divided by the weight of the individual mouse. For each mouse, one kidney was fixed and embedded in paraffin for histological analysis and imaged using methods described above. The other kidney was de-capsulated and macerated for 30 minutes in 5N HCl at 37°C. Tissue was rinsed in deionized water and stored at 4°C overnight. Tubules and glomeruli were dissociated through shaking and suspended in 25 ml of deionized water. Glomeruli were counted on five different 100µl aliquots. This procedure was modified from a published protocol (Phelep et al., 2017). Statistical significance was determined using a two-way ANOVA with R statistical software (version 3.5.1).

Kidneys from 4-month-old mice were collected for RNA isolation and stabilized in RNAlater overnight. Following the same homogenization procedure described above for the skin, kidney RNA was isolated using the RNAeasy Mini Extraction Kit (Qiagen) with on-column DNase digestion. cDNA synthesis was performed using methods described for CD117+ and CD117- cells.

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SUPPLEMENTAL FIGURE LEGENDS

Fig S1. Generation of *Tyr:Cre^{ERT2}, ROSA^{mTmG}* mice. (A) Cross to generate *Tyr:Cre^{ERT2}*, *ROSA^{mTmG}* double heterozygous mice. Mice were injected with tamoxifen once daily for 10 days to generate melanocyte specific EGFP expression. EGFP+ and tdTomato+ cells were sorted using FACS. (B) A Principal component analysis (PCA) plot of RNA-seq data that characterizes trends of the expression profiles of EGFP+ melanocytes (green) and all other skin cells that are tdTomato+ (red). (C) Expression of *Trp63* in EGFP+ and tdTomato+ cells of mouse skin. (D-E) RNA isolated from (D) MNT-1 human melanoma cells and (E) darkly-pigmented human epidermal melanocytes (DP-Mel) was analyzed using RT-qPCR with primers designed to target exon 1 of *MITF-A* and *MITF-M*, n=3. (F) Outline of read junctions used to determine *MITF* isoform abundance from human RNAseq data.

Fig S2. RXR/RAR binding site is conserved between mouse and human. The human *MITF-A* promoter and the murine *Mitf-A* promoter were aligned using the first 1000bp upstream of the transcription start site for the A isoform. A red box indicates the conserved RXR/RAR putative binding site.

Fig S3. Validation of CRISPR/Cas9 knockout of *Mitf* isoforms. (A) IGV visualization of DNA sequencing results showing location of deletion in exon 1A of *Mitf* in *Mitf-A* knockout mice. (B) Comparison of wildtype and knockout *Mitf-A* DNA sequence and resulting amino acid sequence visualized in SnapGene Viewer. Seven-base pair deletion highlighted in wildtype sequence with red box and a red line marks the deletion in the knockout sequence. Red arrows indicate first change in amino acid sequence and the first premature stop codon resulting from CRISPR/Cas9 deletion. (C) Visualization of DNA sequencing results showing location of deletion spanning the splice site for exon 1M of *Mitf* in *Mitf-M* knockout mice.

Fig S4. Consistent coat colors of *Mitf* isoform specific knockout mice. (A-D) Images showing coat color of (A) *Mitf* wildtype mice, (B) *Mitf-A* knockout mice, (C) *Mitf-M* knockout mice, and (D) C57BL/6 albino mice. All mice were imaged on the same day for consistent lighting conditions and are within two weeks of age.

Fig S5. Loss of *Mitf-M* alters pigmentation of tails and paws. (A) Images of *Mitf* wildtype mice showing eyes, paws, and tail. (B) *Mitf-A* knockout mice illustrating eye, paw, and tail pigmentation. (C) Images of *Mitf-M* knockout mice showing loss of pigment in nose, paws, and tails, but retention in the eye. (D) C57BL/6 albino mice illustrating eye, paw, and tail pigmentation.

Fig S6. *Mitf* isoforms play overlapping roles in the kidney. (A) Diagram of kidney region depicted in H&E staining of kidneys at 5X and 20X magnification. (B) Representative images of kidney histology from wildtype mice at 5X magnification (left) and 20X magnification (right). (C) Kidney histology of *Mitf-A*$^{-/-}$ mice. (D) Representative kidney histology from *Mitf-M*$^{-/-}$ mice. (E) Quantification of glomeruli number for males and females of indicated genotypes, n = 3. (F) Relative expression of *Mitf* isoforms in kidneys of indicated mice normalized to wildtype mice. (G) Further comparison of *Mitf* isoform abundance in the kidneys of wildtype and *Mitf* knockout mice.

Fig S7. Loss of *Mitf-M* alters pigmentation of the eye. (A-D) Live animal images of eyes from (A) wildtype, (B) *Mitf-A*$^{-/-}$ mice, (C) *Mitf-M*$^{-/-}$ mice, and (D) albino mice. (E-H) Whole-eye images of iris with pupil indicated (upper) and posterior surface (lower) of (E) wild-type mice, (F) *Mitf-A* knockout mice, (G) *Mitf-M* knockout mice, and (H) albino mice that lack all pigmentation, with reduced pigmentation of the eye in (G). (I) Representative gel showing genotyping at *Crb1* gene for *rd8* allele for 10 mice in both the *Mitf-A* line and the *Mitf-M* line. Arrows denote expected size for wildtype (220bp) and *rd8* (224bp) alleles. (J) Genotyping results from 17 C57BL/6J, 17 C57BL/6N, 51 *Mitf-A* mice, and 50 *Mitf-M* mice. Allele frequencies of wildtype and *rd8 Crb1* alleles were calculated from genotyped mice.

Fig S8. Depigmentation of iris stroma and chorid in *Mitf-M* knockout mice. (A-D) H&E staining focused on the iris and ciliary bodies collected at 20X of (A) wild-type, (B) *Mitf-A* knockout, (C) *Mitf-M* knockout, and (D) albino mice. (E-H) Iris histology showing iris pigment epithelium (above dashed line) and iris stroma (below line) at 63X magnification of (E) wild-type, (F) *Mitf-A*$^{-/-}$ mice, (G) *Mitf-M*$^{-/-}$ mice, and (H) albino mice. (I-L) H&E staining of retinal histology focusing on the choroid and RPE collected at 40X of (I) wild-type mice, (J) *Mitf-A* knockout mice, (K) *Mitf-M* knockout mice, and (L) albino mice.

Fig S9. *Mitf-A* transcripts from *Mitf-A* knockout mice retain deletion. Alignment of Sanger sequencing reads from wild-type and *Mitf-A* knockout mice following amplification. Protein sequence is highlighted in orange under NM_001113198 sequence, with the 7bp deletion highlighted in red.

Table S1. Panel of skin cell markers. RNAseq expression for melanocyte, keratinocyte, fibroblast, and endothelial cell markers.

Table S2. Binding sites in *MITF-M* promoter. Putantive binding sites located in the *MITF-M* promoter identified in MotifMap.

Table S3. Binding sites in *MITF-A* promoter. Putantive binding sites located in the *MITF-A* promoter identified in MotifMap.

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Table S4. List of primers for RT-qPCR and ChIP analysis. Primers designed for chromatin immunoprecitiation (ChIP) and reverse transcriptase-quantitative PCR (qPCR) in human cell lines and mouse tissues.

245 **Table S5. List of genotyping primers.** Genotyping primers for all mouse models used in this study.

Table S6. List of primers and gRNA used for generation of *Mitf* isoform-specific mutant mice.