HHS Public Access

Author manuscript

J Cell Physiol. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

J Cell Physiol. 2019 July ; 234(7): 11780–11791. doi:10.1002/jcp.27840.

BAF60A mediates interactions between MITF and the BRG1-containing SWI/SNF complex during melanocyte differentiation

Shweta Aras[1,2], **Srinivas Vinod Saladi**[1,3], **Tupa Basuroy**[1], **Himangi, G. Marathe**[1,4], **Patrick Lorès**[5], and **Ivana L. de la Serna**[1,*]

[1]Department of Cancer Biology, University of Toledo College of Medicine and Life Sciences, 3035 Arlington Ave, Toledo Ohio 43614, USA

[2]Present address: University of Pennsylvania, Curie Blvd 421, Philadelphia, PA 19104, Philadelphia PA 19140, USA

[3]Present address: Massachusetts General Hospital Cancer Center, GRJ-904, 55 Fruit Street, Boston, MA 02114, USA

[4]Present address: Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA

[5]INSERM U1016, Institut Cochin/CNRS UMR8104/ Universite Paris Descartes, Faculte de Medecine Cochin, 24 rue du Faubourg Saint Jacques, Paris 75014, France

Abstract

SWI/SNF chromatin remodeling enzymes are multi-subunit complexes that contain one of two catalytic subunits, BRG1 or BRM, and 9–11 additional subunits called BAFs. BRG1 interacts with the Microphthalmia-Associated Transcription Factor (MITF) and is required for melanocyte development *in vitro* and *in vivo*. The subunits of SWI/SNF that mediate interactions between BRG1 and MITF have not been elucidated. Three mutually exclusive isoforms of a 60KDa subunit (BAF60A, B, or C) often facilitate interactions with transcription factors during lineage specification. We tested the hypothesis that a BAF60 subunit promotes interactions between MITF and the BRG1-containing SWI/SNF complex. We found that MITF can physically interact with BAF60A, BAF60B, and BAF60C. The interaction between MITF and BAF60A required the basic helix loop helix domain of MITF. Recombinant BAF60A pulled down recombinant MITF, suggesting that the interaction can occur in the absence of other SWI/SNF subunits and other transcriptional regulators of the melanocyte lineage. Depletion of BAF60A in differentiating melanoblasts inhibited melanin synthesis and expression of MITF target genes. MITF promoted BAF60A recruitment to melanocyte specific promoters and BAF60A was required to promote BRG1 recruitment and chromatin remodeling. Thus, BAF60A promotes interactions between MITF and the SWI/SNF complex and is required for melanocyte differentiation.

*Corresponding author: University of Toledo College of Medicine and Life Sciences, Department of Biochemistry and Cancer Biology, 3035 Arlington Ave, Toledo, OH 43614, USA, Phone: (419) 383-4111. FAX: (419) 383-6228. ivana.delaserna@utoledo.edu.

COMPETING FINANCIAL INTEREST STATEMENT

The authors declare that there are no competing financial interests.

Keywords

Melanocyte differentiation; MITF; Chromatin Remodeling; BAF60A

INTRODUCTION

Melanocytes are cells that synthesize melanin and populate the skin, hair follicles, heart, choroid of the eye, and inner ear (Steingrimsson et al., 2004). During embryonic development, the neural crest gives rise to precursors called melanoblasts which then differentiate into melanocytes. Melanocytes in the basal layer of the epidermis protect the skin against the damaging effects of ultraviolet radiation by synthesizing and transferring melanosomes containing melanin to surrounding keratinocytes (Kadekaro et al., 2003). They can transform to melanoma, an aggressive skin cancer with a poor prognosis. Key transcription factors that regulate melanocyte specification, survival, proliferation, and differentiation also have critical functions in melanoma.

The Microphthalmia-Associated Transcription Factor (MITF) is the master regulator of melanocyte differentiation and considered a lineage addiction oncogene in melanoma (Garraway and Sellers, 2006). MITF is a helix loop helix leucine zipper transcription factor that binds to E boxes and regulates expression of genes important for melanocyte differentiation, survival, and proliferation (Levy et al., 2006). MITF target genes include those that encode the tyrosinase family of enzymes required for melanin synthesis: tyrosinase (Tyr), tyrosinase related protein 1 (Tyrp1), and dopachrome tautomerase (Dct) as well as other melanosomal proteins. The Sry-related transcription factor, SOX10 also participates in regulating the expression of these genes (Jiao et al., 2004; Lang et al., 2005; Ludwig et al., 2004; Murisier et al., 2006; Murisier et al., 2007). SOX10 is expressed in neural crest precursors and is critical for establishing the Schwann cell and melanocyte lineages (Wegner, 2005). In melanoblasts, SOX10 activates MITF expression and interacts with MITF to regulate expression of a subset of MITF target genes required for melanin synthesis (Marathe et al., 2017). Transcriptional activation of melanocyte specific genes also involves crosstalk between these lineage specific transcriptional activators and SWI/SNF chromatin remodeling complexes which induce chromatin modifications permissive for transcription (de la Serna et al., 2006).

SWI/SNF enzymes are multi-subunit and evolutionarily conserved chromatin remodeling complexes that utilize energy derived from ATP hydrolysis to physically remodel chromatin structure. The catalytic activity of chromatin remodeling is attributed to two mutually exclusive central ATPases namely BRG1 or BRM which are more than 70% identical to each other (Saladi and de la Serna, 2010). These complexes also contain 8–12 BRG1 or BRM Associated Factors also known as BAFs which can enhance chromatin remodeling activity or interact with cellular factors to help target the complex to genomic loci. Diverse complexes composed of either BRG1 or BRM and an assortment of different BAFs have been detected in cells. Among the ATPases, BRG1 has a predominant role in melanocyte development *in vivo* and differentiation *in vitro* (Laurette et al., 2015; Marathe et al., 2017). A minimal complex consisting of BRG1 and BAF155 or BAF170 has been shown to

functionally interact with zinc finger transcription factors, SP1, EKLF, and GATA1 but not with the helix loop helix transcription factor, TFE3, nor with NF-κB (Kadam et al., 2000; Phelan et al., 1999). Additional BAFs are required to mediate interactions between helix loop helix and many other transcription factors with BRG1 and the SWI/SNF complex (Forcales et al., 2012; Hsiao et al., 2003).

Among the BAFs, one of three variants of a 60KDa subunit, BAF60A, BAF60B, or BAF60C, encoded by Smarcd1, Smarcd2 and Smarcd3 respectively, mediates interactions between the SWI/SNF complex and a number of transcription factors. BAF60A interacts with a diverse set of transcriptional regulators including nuclear hormone receptors, ppaγ-coactivator-1α (PGC-1α), jun/c-fos, p53, and SOX10 (Flajollet et al., 2007; Fryer and Archer, 1998; Hsiao et al., 2003; Ito et al., 2001; Li et al., 2008; Weider et al., 2012). *In vivo*, BAF60A has been studied in liver where conditional deletion of the Smarcd1 gene renders mice resistant to diet-induced-hypercholesteremia and atherosclerosis but does not affect viability(Meng et al., 2015). BAF60B interacts with p53, ATM, and CEBPε (Ji et al., 2017; Priam et al., 2017; Witzel et al., 2017). Deletion of Smarcd2 compromises hematopoietic development and results in late embryonic lethality (Priam et al., 2017; Witzel et al., 2017) BAF60C was shown to interact with the helix loop helix transcription factors, MYOD in myocytes and USF1 in hepatocytes (Simone et al., 2004; Wang et al., 2013). Depletion of BAF60C during mouse development causes heart defects, blocks skeletal muscle differentiation, and results in embryonic lethality (Lickert et al., 2004) Muscle specific deletion of the gene encoding BAF60C impairs glucose sensing and glycolytic metabolism in myotubes (Meng et al., 2013). Both BAF60A and BAF60B were previously found to be in a complex with MITF in melanocytes and melanoma cells (Laurette et al., 2015). BAF60A was also found to be in a complex with SOX10 in melanocytes (Marathe et al., 2017). However, a functional analysis of BAF60A or other BAF60 subunits in the regulation of gene expression in melanocytes has not previously been reported.

In this study, we report that MITF can physically interact with BAF60A, BAF60B, and BAF60C. The interaction between MITF and BAF60A required a region that includes the basic helix loop helix domain of MITF. Recombinant BAF60A pulled down recombinant MITF, suggesting that the interaction can occur in the absence of other components of the SWI/SNF complex and in the absence of SOX10. Depletion of BAF60A in differentiating melanoblasts inhibited melanin synthesis and MITF target genes that regulate melanin synthesis. MITF promoted BAF60A recruitment to a melanocyte specific promoter and BAF60A was required to promote BRG1 recruitment and chromatin remodeling.

MATERIALS AND METHODS

Cell Culture

Melb-a cells were obtained from the Welcome Trust (United Kingdom) and cultured in growth media (RPMI 1640 with 10% fetal bovine serum, 40 picoM fibroblast growth factor and 10ng/ml stem cell factor). Differentiation was induced when cultures were 70% confluent by replacing growth media with differentiation media (DMEM with 10%fetal bovine serum, 2 nM [Nle4, D-Phe7]-alpha melanocyte stimulating hormone (NDP-alpha MSH) and 200 nM phorbol-myristate-acetate). Human Embryonic Kidney (HEK) 293T cells

were obtained from the American Type Culture Collection. They were cultured in DMEM media containing 10% fetal calf serum. 501Mel melanoma cells were cultured as previously described (Keenen et al., 2010).

Plasmids

Vectors for expression of 3X-FLAG-tagged *BAF60* subunits in mammalian cells were previously described (Lores et al., 2010). *Mitf* deletion constructs were generated by PCR and sub-cloned into the Not1/MluI sites of a pCMV-V vector. For bacterial expression, *BAF60A* cDNA was subcloned into the Sal1/HindIII sites of pGEX-2T (GE Healthcare, Pittsburgh, PA, USA) and MITF cDNA was subcloned into the EcoRI/HindIII sites of pRSET-B (Thermofisher, Waltham, MA, USA). All constructs were verified by sequencing.

Cell extracts and immunoblot analysis

Cell extracts were prepared and western blot was performed as described in (Keenen et al., 2010). Antiserum to BRG1 was previously described (de La Serna et al., 2000). The BAF60A antibodies (cat#A301–594 for immunoprecipitations and cat#A301–595 for Westerns) were purchased from Bethyl Laboratories (Montgomery, TX, USA) and previously used in (Wilson et al., 2014). For some experiments, a BAF60A antibody (cat#611728) that was previously used in (Chen et al., 2012) was purchased from BD Biosciences (San Jose, CA). The BAF60B antibody (A301–596) was from Bethyl Laboratories (Montgomery, TX). The BAF60C antibody (ab50556) was purchased from Abcam and previously described in (Goljanek-Whysall et al., 2014). The MITF antibody (ab12039) was also from Abcam (Cambridge, MA, USA) and previously described in (Keenen et al., 2010). The Sox10 (sc17342), TYRP1 (cat#sc10443) and TYR (sc7833) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Tubulin (2148) antibody was from Cell Signaling Technology (Boston, MA, USA) and the FLAG antibody (M2) was from Sigma (St. Louis, MO, USA).

Immunoprecipitations

Melb-a cells were differentiated for 48 hours then lysed and immunoprecipitated with antibodies to BAF60A, BAF60B, and BAF60C. HEK 293T cells were transfected with pCMV or co-transfected with pCMV-MITF-M constructs and 3XFLAG-CMV-BAF60A, B, or C (Lores et al., 2010) using Lipofectamine (LTX) (Thermofisher, Waltham, MA, USA) as described in (Saladi et al., 2013). Cells were lysed after 48 hrs and immunoprecipitated with FLAG antibody as described (Keenen et al., 2010). Elution was conducted with 3X FLAG peptide (Sigma Aldrich, St. Louis, MO, USA) as described in (Fock et al., 2018).

For GST pulldowns, BL21 cells (Thermofisher, Waltham, MA, USA) were transformed with empty pGEX, pGEX-2tBAF60A, or pRSET-B-MITF. Bacterial cultures were induced with IPTG and harvested after 6 hours. pGEX and pGEX-2tBAF60A were lysed and immunoprecipitated with glutathione agarose (Sigma Aldrich, St. Louis, MO, USA). The washed beads were then incubated with extracts from pRSET-B-MITF transformed cells and immunoprecipitated.

RNA Isolation and Quantitative real time PCR

Total RNA was isolated using Trizol (Invitrogen, Waltham, MA, USA) and cDNA was prepared using the Quantitect Reverse Transcription kit (Qiagen, Valencia, CA, USA). Quantitative PCR (qPCR) was performed as previously described (Keenen et al., 2010).

Primer sequences to mouse genes were*: Baf60a*: 5'-GGA AGC TGC GAA TTT TCA TT −3'and 5'-TTT GTC CAG TTC GAT CAC CA-3', *Mitf*: 5'-CAG ACC CAC CTG GAA AAC C-3' and 5'-ATG GTG AGC TCA GGA CTT GG-3', *Tyr*: 5'-TTC AAA GGG GTG GAT GAC CG-3' and 5'-GAC ACA TAG TAA TGC ATC C-3', *Tyrp1*: 5'-GCC CCA ACT CTG TCT TTT CTC AAT-3' and 5'-GAT CGG CGT TAT ACC TCC TTA GC-3', *Dct*: 5'-GGA CCG GCC CCG ACT GTA ATC-3' and 5'-GTA GGG CAA CGC AAA GGA CTC AT-3', *Trpm1*: 5'-CCT ACG ACA CCA AGC CAG AT-3', and 5'-GAC GAC ACC AGT GCT CAC AC-3' Rpl7: 5'-GGA GGA AGC TCA TCT ATG AGA AGG-3' and 5'-AAG ATC TGT GGA AGA GGA AGG AGC-3'. Primer sequences to human genes were: *BAF60A*: 5'-GTA TGG GCC AGA CAA CCA TG-3' and 5'-ACG AGT CTG GGT ATG GAT GC-3', *MITF*: 5'-CTC GAG CTC ATG GAC TTT CC-3' and CCA GTT CCG AGG TTG TTG TT-3', *TYR*: 5'-GGT GGG AAC AAG AAA TCC AG-3' and 5'-TCC TCC AAT CGG CTA CAG AC-3', *TYRP1*: 5'-TGG GAT CCA GAA GCA ACT TT-3' and 5'-TGT GGT TCA GGA AGA CGT TG-3', *DCT*: 5'-GGG TTT CTG CTC AGT TGC TT-3' and GGA TGT AGG GAC CAC TCC AG-3', *TRPM1*: TGC TCC ATC TCA TGG TGA AA-3' and 5'-TCC CCT ACG TGG CTG ATA AC-3', *RPL19*: 5'-AAA CAA GCG GAT TCT CAT GG-3' and 5'-TTG GTC TCT TCC TCC TTG GAT-3'

siRNA Knockdown

Smart pools of siRNA targeting mouse and human BAF60A were obtained from Dharmacon (Waltham, MA, USA). Additional siRNAs targeting BAF60A were obtained from Integrated DNA Technologies (Coralville, Iowa) Undifferentiated Melb-a cells were transfected in growth medium for 48 hours. The medium was then replaced and the cells were cultured in differentiation medium for an additional 48 hours. 501 Mel cells were cultured and transfected as previously described (Keenen et al., 2010).

Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE)

FAIRE was performed as described in (Simon et al., 2012) with some modifications. Cells were crosslinked in 1% formaldehyde for 6 minutes at room temperature and quenched with 125mM glycine. Cells were then lysed and sonicated as described in (Keenen et al., 2010). Sonicated chromatin was subjected to two rounds of phenol/chloroform extraction, back extracted once with TE and then once with chloroform. The aqueous phase was ethanol precipitated and digested with 0.2mg/ml Proteinase K for one hour at 55°C. Cross-links were then reversed by heating overnight at 65°C. DNA was then purified by an additional phenol chloroform extraction and ethanol precipitation. Control Inputs were 10% of each sample that was heated at 65°C overnight to reverse crosslinking prior to purification. The primers used were: mouse *Tyr* proximal: 5'-AGT CAT GTG CTT TGC AGA AGA T-3' and 5'-CAG CCA AGA ACA TTT TCT CCT T-3', mouse *Tyrp1* proximal region: 5'-GCA AAA TCT CTT CAG CGT CTC-3' and 5'-AGC CAG ATT CCT CAC ACT GG-3', mouse, *IgH*

Enhancer: 5’-GCC GAT CAG AAC CAG AAC ACC-3’ and 5’-TGG TGG GGC TGG ACA GAG TGT TTC-3’.

Chromatin immunoprecipitation (ChIP)

ChIP was performed as described (Keenen et al., 2010) using the same antibodies as in Westerns. Primers were the same as used in FAIRE. The primer sequences for mouse genes were: *Tyrp1* promoter (−162 to +65): (forward: 5’-GCAAAATCTCTTCAGCGTCTC-3’) and (reverse: 5’-AGCCAGATTCCTCACACTGG-3’), *Tyr* promoter (−254 to −56): (forward: 5’-AGTCATGTGCTTTGCAGAAGAT −3’) and (reverse: 5’-CAGCCAAGAACATTTTCTCCTT-3’) and *Igh* enhancer (forward: 5’-GCCGATCAGAACCAGAACACC-3’) and (reverse: 5—TGGTGGGCTGGACAGAGTGTTTC-3’)

Propidium iodide (PI) staining and fluorescence-activated cell sorting (FACS)

Cells were fixed with 100% ethanol for 1 h, stained with PI-RNAse solution for 30 minutes, and loaded on a FACS-Calibur (BD Biosciences, San Jose, CA, USA) by the University of Toledo Flow Cytometry Core Facility. Data were analyzed using Cell Quest Pro (BD Biosciences).

Melanin Quantification

Melanin was quantified as previously described (Marathe et al., 2017). Cells were counted and then lysed in 0.1 M NaOH at 37^{o}C with vortexing for 20 min. Melanin content was calculated based on the absorbance at 475 nm as compared to the standard curve obtained using synthetic melanin (Sigma, St. Louis, MO, USA) after normalizing cell numbers.

Statistical analysis

Statistical significance was calculated by the Student’s *t* test when comparing two sets of data and a one way ANOVA followed by Dunnette’s multiple comparison tests for comparing more than two sets of data (Graphpad Prism, version 5.03).

RESULTS

BAF60 subunits are expressed in differentiating Melb-a cells and interact with MITF

Melb-a cells are derived from the epidermis of neonatal mice and can be cultured as unpigmented melanoblasts or they can be induced to differentiate into pigmented melanocytes (Sviderskaya et al., 2001; Sviderskaya et al., 1995). In order to begin to evaluate the function of the three BAF60 isoforms in melanocyte differentiation, we assessed expression of BAF60A, BAF60B, and BAF60C in differentiating Melb-a melanoblasts. As previously reported (Marathe et al., 2017), we found that pigmentation became visible after approximately 48 hours of differentiation (Fig. 1A top). BRG1 levels were fairly constant during differentiation while MITF was expressed at a low level prior to differentiation and increased 12 hours after differentiation. A melanogenic marker, TYRP1, became detectable after 24 hours and increased substantially by 48 hours, coordinately with the change in visible pigmentation (Fig.1A bottom). We found that all three BAF60 subunits were

expressed in both undifferentiated and differentiated Melb-a cells. BAF60A expression increased modestly 12 hours after differentiation while BAF60B and BAF60C levels changed less than two fold through the time period examined.

We performed co-immunoprecipitation experiments with antibodies to the BAF60 subunits to determine if any of them interact with MITF. We detected a strong interaction between MITF and BAF60A and a somewhat weaker interaction between MITF and BAF60C. The interaction between MITF and BAF60B was barely above the IgG control (Fig. 1B). In reciprocal experiments, MITF was used to immunoprecipitate the BAF60 subunits in undifferentiated and differentiating Melb-a cells. These experiments confirm strong interactions between MITF and BAF60A in both undifferentiated and differentiating Melb-a cells (Fig.1C). Considerably weaker interactions were detected between MITF and BAF60B and BAF60C.

MITF interacts with BAF60A through its basic helix loop helix (bHLH) domain

To further characterize the physical interactions between the BAF60 isoforms and MITF, HEK 293T cells were co-transfected with expression vectors for MITF and 3X FLAG-tagged BAF60A, B or C. Immunoprecipitations were performed with an antibody directed against the FLAG epitope of BAF60 proteins. As shown in Figure 2A, BRG1, which forms a central ATPase of the SWI/SNF complex was co-immunoprecipitaed with all three BAF60 proteins indicating that they each form a SWI/SNF complex containing BRG1. MITF also co-immunoprecipitated with all three BAF60 proteins. Thus, although MITF preferentially interacts with BAF60A in Melb-a cells (Fig. 1B and 1C), MITF also strongly interacts with the other BAF60 subunits in 293T cells.

We proceeded to define the region of MITF that mediates the interaction with BAF60A by generating MITF C-terminal deletion constructs (Figure 2B). HEK 293T cells were co-transfected with wild type or MITF deletion constructs and 3X-FLAG tagged BAF60A. C-terminal deletion constructs which had a portion of the C terminal transactivation domain of MITF (Takeda et al., 2000) removed or those that completely lacked this domain retained the ability to interact with BAF60A. However, further deletion of the C terminus to encompass the bHLH region of MITF abolished the interaction with BAF60A, indicating its requirement (Fig. 2B). We performed GST-pulldowns from proteins that were purified from bacterial cells, and found that the two proteins interact, even in the absence of other SWI/SNF components and SOX10, a neural crest transcription factor that was previously demonstrated to interact with BAF60A (Marathe et al., 2017; Weider et al., 2012) (Fig. 2C). Thus, we identify the BAF60A subunit of the SWI/SNF complex as an interacting partner of MITF.

Depletion of BAF60A in Melb-a melanoblasts abrogates melanin synthesis and modestly affects cell cycle progression

Melb-a cells synthesize melanin and become pigmented when induced to differentiate (Sviderskaya et al., 2001). MITF plays a crucial role in regulating genes involved in melanin synthesis. Since BAF60A interacts with MITF, we investigated whether it is required for MITF-regulated processes. Transfection of Melb-a cells with siRNAs targeting BAF60A

depleted BAF60A but did not decrease expression of BAF60B or BAF60C, BRG1, nor SOX10 (Fig. 3A). However, BAF60A depletion resulted in decreased levels of MITF and two enzymes involved in melanin synthesis, Tyrosinase (TYR) and Tyrosinase related protein-1(TYRP1). The expression of the genes encoding these enzymes is regulated by MITF and is an indicator of melanocyte differentiation. Consistent with decreased TYR and TYRP1 levels, depletion of BAF60A resulted in cells that were visibly lighter (Fig. 3B, top) and that synthesized less melanin (Fig. 3B, bottom).

Cellular differentiation is characterized by decreased proliferation and arrest in the G1 phase of the cell cycle, which in melanocytes is promoted by MITF (Carreira et al., 2005). We found that depletion of BAF60A resulted in a modest but statistically significant increase in the percent of cells in S phase and a decrease in the percent of cells in the G1 phase of the cell cycle (Fig. 3C). This modest change in the cell cycle profile may contribute to the decrease in melanocyte differentiation or may be a result of the decrease in differentiation that we observed upon depletion of BAF60A.

Depletion of BAF60A abrogates expression of MITF and MITF target genes involved in melanin synthesis

We assessed the effects of theBAF60A knockdown on the expression of Mitf and several Mitf target genes at the mRNA level by transfecting Melb-a cells with control siRNA, a smart pool of siRNA that targets BAF60A (Fig. 4A, left) or two additional siRNAs that target BAF60A (Fig. 4A, right). Knockdown of BAF60A significantly abrogated the expression of Mitf, Tyr, and Tyrp1 and two other MITF target genes, dopachrome tautomerase (Dct), and Trpm1 in mouse Melb-a cells (Fig.4B). A previous report found that BAF60A forms a complex with MITF in 501Mel melanoma cells (Laurette et al., 2015). However, a functional requirement for BAF60A in the regulation of MITF target genes was not established in 501Mel cells. We found that depletion of BAF60A in 501Mel cells (Fig. 4C) also abrogates MITF and MITF target gene expression (Fig. 4D). Therefore, BAF60A is required for expression of melanocyte specific genes in differentiating mouse melanoblasts and in human melanoma cells.

MITF is required for BAF60A recruitment to the Tyr and Tyrp1 promoters during melanocyte differentiation

We performed chromatin immunoprecipitation (ChIP) in undifferentiated and differentiated Melb-a cells to determine whether BAF60A is recruited to promoters regulated by MITF during melanocyte differentiation. We found that although initially low on the *Tyrp1* and *Tyr* promoters in undifferentiated cells, BAF60A occupancy increases upon differentiation (Fig. 5A). To determine if BAF60A recruitment is dependent on MITF, we depleted MITF using siRNA (Fig. 5B). ChIP analysis indicated that depletion of MITF significantly reduces BAF60A enrichment on the *Tyrp1* and *Tyr* promoters (Fig. 5C). Thus, MITF is required for BAF60A recruitment to the promoters of melanocyte specific genes during melanocyte differentiation.

BAF60A is required for BRG1 recruitment to the Tyr and Tyrp1 promoters

To investigate the requirement for BAF60A in the transcriptional regulation of MITF target promoters, we performed ChIP to determine if BAF60A depletion compromises MITF and BRG1 binding. We found that BAF60A depletion had a small effect on MITF binding to the *Tyrp1* and *Tyr* promoters that was statistically significant only on the *Tyr* promoter (Fig. 6A). In contrast, BAF60A depletion significantly abrogated BRG1 recruitment to the *Tyrp1* and *Tyr* promoters (Fig. 6B). Thus, BRG1 recruitment is dependent on BAF60A.

BAF60A is required for chromatin remodeling on the Tyr and Tyrp1 promoters

We previously found that chromatin accessibility at the Tyr and Tyrp1promoters increases in an MITF and BRG1 dependent manner as Melb-a cells are differentiated (Marathe et al., 2017). To determine if BAF60A is also required for chromatin remodeling, we performed Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) with control and BAF60A depleted cells. Consistent with a requirement for BAF60A in the recruitment of the BRG1 catalytic subunit of the SWI/SNF complex, we found that depletion of BAF60A significantly reduced chromatin accessibility at the *Tyrp1* and *Tyr* promoters (Fig. 7).

DISCUSSION

The SWI/SNF complex has previously been implicated in melanocyte development and melanoma proliferation. Several studies show that the catalytic subunit of the complex, BRG1, is essential for both processes (Keenen et al., 2010; Laurette et al., 2015; Marathe et al., 2017; Vachtenheim et al., 2010). BRG1 is recruited to the regulatory regions of melanocyte specific genes by the master regulator of melanocyte development, MITF, and co-activates target genes by remodeling chromatin structure. Although previous studies have co-immunoprecipitated BRG1-associated factors (BAFs) with MITF, there have not yet been any studies to investigate the functional role of any of these associated factors in melanocytic cells. Especially lacking is the identification of subunits that mediate direct interactions between MITF and the SWI/SNF complex and function to promote melanocyte differentiation.

The three BAF60 isoforms are mutually exclusive components of SWI/SNF complexes that mediate direct interactions with master regulators of differentiation in a lineage specific manner (Puri and Mercola, 2012). In this study, we found that the BAF60A, BAF60B, and BAF60C are all expressed in melanocytes and physically interact with MITF. We pursued studies to further characterize the interaction between MITF and BAF60A because we found that depletion of BAF60A by RNAi had the greatest suppressive effect on the MITF target genes assayed.

The interaction between MITF and BAF60A required the helix loop helix domain of MITF and occurred between proteins purified from bacterial cells, suggesting that BAF60A mediates direct interactions with MITF. To our knowledge, this is the first report that BAF60A can interact with other helix loop helix transcription factors. Like MITF, MYOD, the master regulator of muscle differentiation, also has a helix loop helix domain but BAF60A is not highly expressed in muscle (Forcales et al., 2012). Previous studies in

muscle indicate that both BAF60B and C interact with MYOD but that BAF60C is the critical subunit required for expression of a majority of MYOD target genes. Indeed, over-expression of BAF60A and to a lesser extent, BAF60B, in muscle progenitors impedes muscle differentiation by effectively diminishing BAF60C incorporation into the SWI/SNF complex (Saccone et al., 2014). Interestingly, BAF60C also promotes hepatic cell differentiation while BAF60B plays a negative role by blocking lineage conversion (Ji et al., 2017). In contrast, BAF60A promotes endoderm differentiation and restricts embryonic cell pluripotency (Alajem et al., 2015). BAF60A is the only BAF60 subunit to interact with SOX10 in Schwann cells (Weider et al., 2012). Thus, although lineages often express two or more of the BAF60 subunits, one subunit seems to play a predominant role in the differentiation of a particular cell type. We found that BAF60A is required to promote melanocyte differentiation and melanin synthesis.

Our studies indicate that BAF60A promotes interactions between MITF and the SWI/SNF complex because depletion of BAF60A compromised the recruitment of BRG1 to the promoters of MITF-regulated pigmentation genes and decreased chromatin accessibility on those promoters (Fig. 8). We also detected a small decrease in MITF occupancy on the TYR and TYRP1 promoters. This may reflect the decrease in MITF levels when BAF60A is depleted or it may suggest that BAF60A facilitates MITF binding. A two-step model whereby SWI/SNF components facilitate MYOD binding through interactions with a pioneer transcription factor was demonstrated to occur during muscle differentiation (de la Serna et al., 2005; Forcales et al., 2012) and could potentially explain how MITF initially gains access to its binding sites in repressive chromatin structure during melanocyte differentiation. However, since the decrease in MITF binding is small, a different SWI/SNF subunit or even a different chromatin remodeling enzyme may fulfill this role in melanocytes.

In conclusion, our studies indicate that BAF60A plays a central role in melanocyte differentiation by interacting with MITF to promote expression of pigmentation genes. Although BAF60B and BAF60C also interact with MITF when over-expressed in 293T cells, the interactions between MITF and BAF60A were markedly stronger than the interactions between MITF and BAF60B or BAF60C in Melb-a cells. Furthermore, knockdown of BAF60A had noticeable effects on pigmentation and on melanocyte specific gene expression. Thus, our data suggest that BAF60A is the critical BAF60 subunit for melanocyte lineage specification. However, although BAF60C interacted weakly with MITF in Melb-a cells, Melba-cells transfected with siRNA targeting BAF60C were visibly lighter than controls, suggesting that BAF60C can also contribute to the differentiation process (supplementary Fig. 1). Since MITF is not the only transcription factor (Seberg et al., 2017) to contribute to melanocyte development, we do not rule out a role for BAF60C in the regulation of pigmentation genes through interactions with other transcription factors involved in melanocyte specification. Furthermore, since all three BAFs strongly interact with MITF when over-expressed in 293T cells, the role of each BAF may be different in other contexts such as melanoma. Indeed, an unbiased screen identified BAF60B as an MITF interacting protein in melanoma cells (Laurette et al., 2015).

In addition to pigmentation genes, MITF regulates genes involved in the cell cycle, survival, the DNA damage response, and energy metabolism (Cheli et al., 2010; Giuliano et al., 2010; Slade and Pulinilkunnil, 2017). These MITF functions are especially important in melanoma where MITF is considered a lineage specific oncogene, having what often appears to be paradoxical roles at different stages of the oncogenic process. It is not well understood how MITF appropriately regulates such a diverse set of genes in response to extracellular signals. Dynamic interactions with diverse SWI/SNF sub-complexes, generated by combinatorial assembly of alternative subunits such as the BAF60 isoforms, may help MITF to regulate discrete programs of gene expression by forming a "code" that impinges upon the function of other transcriptional regulators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: Contract grant sponsor: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS); Contract Grant Number: R01(ARO59379) and University of Toledo Bridge Funding

REFERENCES

Alajem A, Biran A, Harikumar A, Sailaja BS, Aaronson Y, Livyatan I, Nissim-Rafinia M, Sommer AG, Mostoslavsky G, Gerbasi VR, Golden DE, Datta A, Sze SK, Meshorer E. 2015 Differential association of chromatin proteins identifies BAF60a/SMARCD1 as a regulator of embryonic stem cell differentiation. Cell reports 10(12):2019–2031. [PubMed: 25818293]

Carreira S, Goodall J, Aksan I, La Rocca SA, Galibert MD, Denat L, Larue L, Goding CR. 2005 Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature 433(7027):764–769. [PubMed: 15716956]

Cheli Y, Ohanna M, Ballotti R, Bertolotto C. 2010 Fifteen-year quest for microphthalmia-associated transcription factor target genes. Pigment Cell Melanoma Res 23(1):27–40. [PubMed: 19995375]

Chen L, Fulcoli FG, Ferrentino R, Martucciello S, Illingworth EA, Baldini A. 2012 Transcriptional control in cardiac progenitors: Tbx1 interacts with the BAF chromatin remodeling complex and regulates Wnt5a. PLoS Genet 8(3):e1002571. [PubMed: 22438823]

de La Serna IL, Carlson KA, Hill DA, Guidi CJ, Stephenson RO, Sif S, Kingston RE, Imbalzano AN. 2000 Mammalian SWI-SNF complexes contribute to activation of the hsp70 gene. Mol Cell Biol 20(8):2839–2851. [PubMed: 10733587]

de la Serna IL, Ohkawa Y, Berkes CA, Bergstrom DA, Dacwag CS, Tapscott SJ, Imbalzano AN. 2005 MyoD targets chromatin remodeling complexes to the myogenin locus prior to forming a stable DNA-bound complex. Mol Cell Biol 25(10):3997–4009. [PubMed: 15870273]

de la Serna IL, Ohkawa Y, Higashi C, Dutta C, Osias J, Kommajosyula N, Tachibana T, Imbalzano AN. 2006 The microphthalmia-associated transcription factor requires SWI/SNF enzymes to activate melanocyte-specific genes. J Biol Chem 281(29):20233–20241. [PubMed: 16648630]

Flajollet S, Lefebvre B, Cudejko C, Staels B, Lefebvre P. 2007 The core component of the mammalian SWI/SNF complex SMARCD3/BAF60c is a coactivator for the nuclear retinoic acid receptor. Mol Cell Endocrinol 270(1–2):23–32. [PubMed: 17363140]

Fock V, Gudmundsson SR, Gunnlaugsson HO, Stefansson JA, Ionasz V, Schepsky A, Viarigi J, Reynisson IE, Pogenberg V, Wilmanns M, Ogmundsdottir MH, Steingrimsson E. 2018 Subcellular localization and stability of MITF are modulated by the bHLH-Zip domain. Pigment Cell Melanoma Res.

Forcales SV, Albini S, Giordani L, Malecova B, Cignolo L, Chernov A, Coutinho P, Saccone V, Consalvi S, Williams R, Wang K, Wu Z, Baranovskaya S, Miller A, Dilworth FJ, Puri PL. 2012

Signal-dependent incorporation of MyoD-BAF60c into Brg1-based SWI/SNF chromatin-remodelling complex. Embo J 31(2):301–316. [PubMed: 22068056]

Fryer CJ, Archer TK. 1998 Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. Nature 393(6680):88–91. [PubMed: 9590696]

Garraway LA, Sellers WR. 2006 Lineage dependency and lineage-survival oncogenes in human cancer. Nat Rev Cancer 6(8):593–602. [PubMed: 16862190]

Giuliano S, Cheli Y, Ohanna M, Bonet C, Beuret L, Bille K, Loubat A, Hofman V, Hofman P, Ponzio G, Bahadoran P, Ballotti R, Bertolotto C. 2010 Microphthalmia-associated transcription factor controls the DNA damage response and a lineage-specific senescence program in melanomas. Cancer Res 70(9):3813–3822. [PubMed: 20388797]

Goljanek-Whysall K, Mok GF, Fahad Alrefaei A, Kennerley N, Wheeler GN, Munsterberg A. 2014 myomiR-dependent switching of BAF60 variant incorporation into Brg1 chromatin remodeling complexes during embryo myogenesis. Development 141(17):3378–3387. [PubMed: 25078649]

Hsiao PW, Fryer CJ, Trotter KW, Wang W, Archer TK. 2003 BAF60a mediates critical interactions between nuclear receptors and the BRG1 chromatin-remodeling complex for transactivation. Mol Cell Biol 23(17):6210–6220. [PubMed: 12917342]

Ito T, Yamauchi M, Nishina M, Yamamichi N, Mizutani T, Ui M, Murakami M, Iba H. 2001 Identification of SWI.SNF complex subunit BAF60a as a determinant of the transactivation potential of Fos/Jun dimers. J Biol Chem 276(4):2852–2857. [PubMed: 11053448]

Ji S, Zhu L, Gao Y, Zhang X, Yan Y, Cen J, Li R, Zeng R, Liao L, Hou C, Gao Y, Gao S, Wei G, Hui L. 2017 Baf60b-mediated ATM-p53 activation blocks cell identity conversion by sensing chromatin opening. Cell Res 27(5):642–656. [PubMed: 28303890]

Jiao Z, Mollaaghababa R, Pavan WJ, Antonellis A, Green ED, Hornyak TJ. 2004 Direct interaction of Sox10 with the promoter of murine Dopachrome Tautomerase (Dct) and synergistic activation of Dct expression with Mitf. Pigment Cell Res 17(4):352–362. [PubMed: 15250937]

Kadam S, McAlpine GS, Phelan ML, Kingston RE, Jones KA, Emerson BM. 2000 Functional selectivity of recombinant mammalian SWI/SNF subunits. Genes Dev 14(19):2441–2451. [PubMed: 11018012]

Kadekaro AL, Kavanagh RJ, Wakamatsu K, Ito S, Pipitone MA, Abdel-Malek ZA. 2003 Cutaneous photobiology. The melanocyte vs. the sun: who will win the final round? Pigment Cell Res 16(5): 434–447. [PubMed: 12950718]

Keenen B, Qi H, Saladi SV, Yeung M, de la Serna IL. 2010 Heterogeneous SWI/SNF chromatin remodeling complexes promote expression of microphthalmia-associated transcription factor target genes in melanoma. Oncogene 29(1):81–92. [PubMed: 19784067]

Lang D, Lu MM, Huang L, Engleka KA, Zhang M, Chu EY, Lipner S, Skoultchi A, Millar SE, Epstein JA. 2005 Pax3 functions at a nodal point in melanocyte stem cell differentiation. Nature 433(7028):884–887. [PubMed: 15729346]

Laurette P, Strub T, Koludrovic D, Keime C, Le Gras S, Seberg H, Van Otterloo E, Imrichova H, Siddaway R, Aerts S, Cornell RA, Mengus G, Davidson I. 2015 Transcription factor MITF and remodeller BRG1 define chromatin organisation at regulatory elements in melanoma cells. eLife 4.

Levy C, Khaled M, Fisher DE. 2006 MITF: master regulator of melanocyte development and melanoma oncogene. Trends Mol Med 12(9):406–414. [PubMed: 16899407]

Li S, Liu C, Li N, Hao T, Han T, Hill DE, Vidal M, Lin JD. 2008 Genome-wide coactivation analysis of PGC-1alpha identifies BAF60a as a regulator of hepatic lipid metabolism. Cell Metab 8(2):105–117. [PubMed: 18680712]

Lickert H, Takeuchi JK, Von Both I, Walls JR, McAuliffe F, Adamson SL, Henkelman RM, Wrana JL, Rossant J, Bruneau BG. 2004 Baf60c is essential for function of BAF chromatin remodelling complexes in heart development. Nature 432(7013):107–112. [PubMed: 15525990]

Lores P, Visvikis O, Luna R, Lemichez E, Gacon G. 2010 The SWI/SNF protein BAF60b is ubiquitinated through a signalling process involving Rac GTPase and the RING finger protein Unkempt. The FEBS journal 277(6):1453–1464. [PubMed: 20148946]

Ludwig A, Rehberg S, Wegner M. 2004 Melanocyte-specific expression of dopachrome tautomerase is dependent on synergistic gene activation by the Sox10 and Mitf transcription factors. FEBS Lett 556(1–3):236–244. [PubMed: 14706856]

Marathe HG, Watkins-Chow DE, Weider M, Hoffmann A, Mehta G, Trivedi A, Aras S, Basuroy T, Mehrotra A, Bennett DC, Wegner M, Pavan WJ, de la Serna IL. 2017 BRG1 interacts with SOX10 to establish the melanocyte lineage and to promote differentiation. Nucleic Acids Res 45(11): 6442–6458. [PubMed: 28431046]

Meng ZX, Li S, Wang L, Ko HJ, Lee Y, Jung DY, Okutsu M, Yan Z, Kim JK, Lin JD. 2013 Baf60c drives glycolytic metabolism in the muscle and improves systemic glucose homeostasis through Deptor-mediated Akt activation. Nat Med 19(5):640–645. [PubMed: 23563706]

Meng ZX, Wang L, Chang L, Sun J, Bao J, Li Y, Chen YE, Lin JD. 2015 A Diet-Sensitive BAF60a-Mediated Pathway Links Hepatic Bile Acid Metabolism to Cholesterol Absorption and Atherosclerosis. Cell reports 13(8):1658–1669. [PubMed: 26586440]

Murisier F, Guichard S, Beermann F. 2006 A conserved transcriptional enhancer that specifies Tyrp1 expression to melanocytes. Dev Biol 298(2):644–655. [PubMed: 16934245]

Murisier F, Guichard S, Beermann F. 2007 The tyrosinase enhancer is activated by Sox10 and Mitf in mouse melanocytes. Pigment Cell Res 20(3):173–184. [PubMed: 17516925]

Phelan ML, Sif S, Narlikar GJ, Kingston RE. 1999 Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol Cell 3(2):247–253. [PubMed: 10078207]

Priam P, Krasteva V, Rousseau P, D'Angelo G, Gaboury L, Sauvageau G, Lessard JA. 2017 SMARCD2 subunit of SWI/SNF chromatin-remodeling complexes mediates granulopoiesis through a CEBPvarepsilon dependent mechanism. Nat Genet 49(5):753–764. [PubMed: 28369034]

Puri PL, Mercola M. 2012 BAF60 A, B, and Cs of muscle determination and renewal. Genes Dev 26(24):2673–2683. [PubMed: 23222103]

Saccone V, Consalvi S, Giordani L, Mozzetta C, Barozzi I, Sandona M, Ryan T, Rojas-Munoz A, Madaro L, Fasanaro P, Borsellino G, De Bardi M, Frige G, Termanini A, Sun X, Rossant J, Bruneau BG, Mercola M, Minucci S, Puri PL. 2014 HDAC-regulated myomiRs control BAF60 variant exchange and direct the functional phenotype of fibro-adipogenic progenitors in dystrophic muscles. Genes Dev 28(8):841–857. [PubMed: 24682306]

Saladi SV, de la Serna IL. 2010 ATP dependent chromatin remodeling enzymes in embryonic stem cells. Stem Cell Rev 6(1):62–73. [PubMed: 20148317]

Saladi SV, Wong PG, Trivedi AR, Marathe HG, Keenen B, Aras S, Liew ZQ, Setaluri V, de la Serna IL. 2013 BRG1 promotes survival of UV-irradiated melanoma cells by cooperating with MITF to activate the melanoma inhibitor of apoptosis gene. Pigment Cell Melanoma Res 26(3):377–391. [PubMed: 23480510]

Seberg HE, Van Otterloo E, Cornell RA. 2017 Beyond MITF: Multiple transcription factors directly regulate the cellular phenotype in melanocytes and melanoma. Pigment Cell Melanoma Res 30(5): 454–466. [PubMed: 28649789]

Simon JM, Giresi PG, Davis IJ, Lieb JD. 2012 Using formaldehyde-assisted isolation of regulatory elements (FAIRE) to isolate active regulatory DNA. Nat Protoc 7(2):256–267. [PubMed: 22262007]

Simone C, Forcales SV, Hill DA, Imbalzano AN, Latella L, Puri PL. 2004 p38 pathway targets SWI-SNF chromatin-remodeling complex to muscle-specific loci. Nat Genet 36(7):738–743. [PubMed: 15208625]

Slade L, Pulinilkunnil T. 2017 The MiTF/TFE Family of Transcription Factors: Master Regulators of Organelle Signaling, Metabolism, and Stress Adaptation. Mol Cancer Res 15(12):1637–1643. [PubMed: 28851811]

Steingrimsson E, Copeland NG, Jenkins NA. 2004 Melanocytes and the microphthalmia transcription factor network. Annu Rev Genet 38:365–411. [PubMed: 15568981]

Sviderskaya EV, Hill SP, Balachandar D, Barsh GS, Bennett DC. 2001 Agouti signaling protein and other factors modulating differentiation and proliferation of immortal melanoblasts. Dev Dyn 221(4):373–379. [PubMed: 11500974]

Sviderskaya EV, Wakeling WF, Bennett DC. 1995 A cloned, immortal line of murine melanoblasts inducible to differentiate to melanocytes. Development 121(5):1547–1557. [PubMed: 7540532]

Takeda K, Yasumoto K, Takada R, Takada S, Watanabe K, Udono T, Saito H, Takahashi K, Shibahara S. 2000 Induction of melanocyte-specific microphthalmia-associated transcription factor by Wnt-3a. J Biol Chem 275(19):14013–14016. [PubMed: 10747853]

Vachtenheim J, Ondrusova L, Borovansky J. 2010 SWI/SNF chromatin remodeling complex is critical for the expression of microphthalmia-associated transcription factor in melanoma cells. Biochem Biophys Res Commun 392(3):454–459. [PubMed: 20083088]

Wang Y, Wong RH, Tang T, Hudak CS, Yang D, Duncan RE, Sul HS. 2013 Phosphorylation and recruitment of BAF60c in chromatin remodeling for lipogenesis in response to insulin. Mol Cell 49(2):283–297. [PubMed: 23219531]

Wegner M 2005 Secrets to a healthy Sox life: lessons for melanocytes. Pigment Cell Res 18(2):74–85. [PubMed: 15760336]

Weider M, Kuspert M, Bischof M, Vogl MR, Hornig J, Loy K, Kosian T, Muller J, Hillgartner S, Tamm ER, Metzger D, Wegner M. 2012 Chromatin-remodeling factor Brg1 is required for Schwann cell differentiation and myelination. Dev Cell 23(1):193–201. [PubMed: 22814607]

Wilson BG, Helming KC, Wang X, Kim Y, Vazquez F, Jagani Z, Hahn WC, Roberts CW. 2014 Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation. Mol Cell Biol 34(6):1136–1144. [PubMed: 24421395]

Witzel M, Petersheim D, Fan Y, Bahrami E, Racek T, Rohlfs M, Puchalka J, Mertes C, Gagneur J, Ziegenhain C, Enard W, Stray-Pedersen A, Arkwright PD, Abboud MR, Pazhakh V, Lieschke GJ, Krawitz PM, Dahlhoff M, Schneider MR, Wolf E, Horny HP, Schmidt H, Schaffer AA, Klein C. 2017 Chromatin-remodeling factor SMARCD2 regulates transcriptional networks controlling differentiation of neutrophil granulocytes. Nat Genet 49(5):742–752. [PubMed: 28369036]

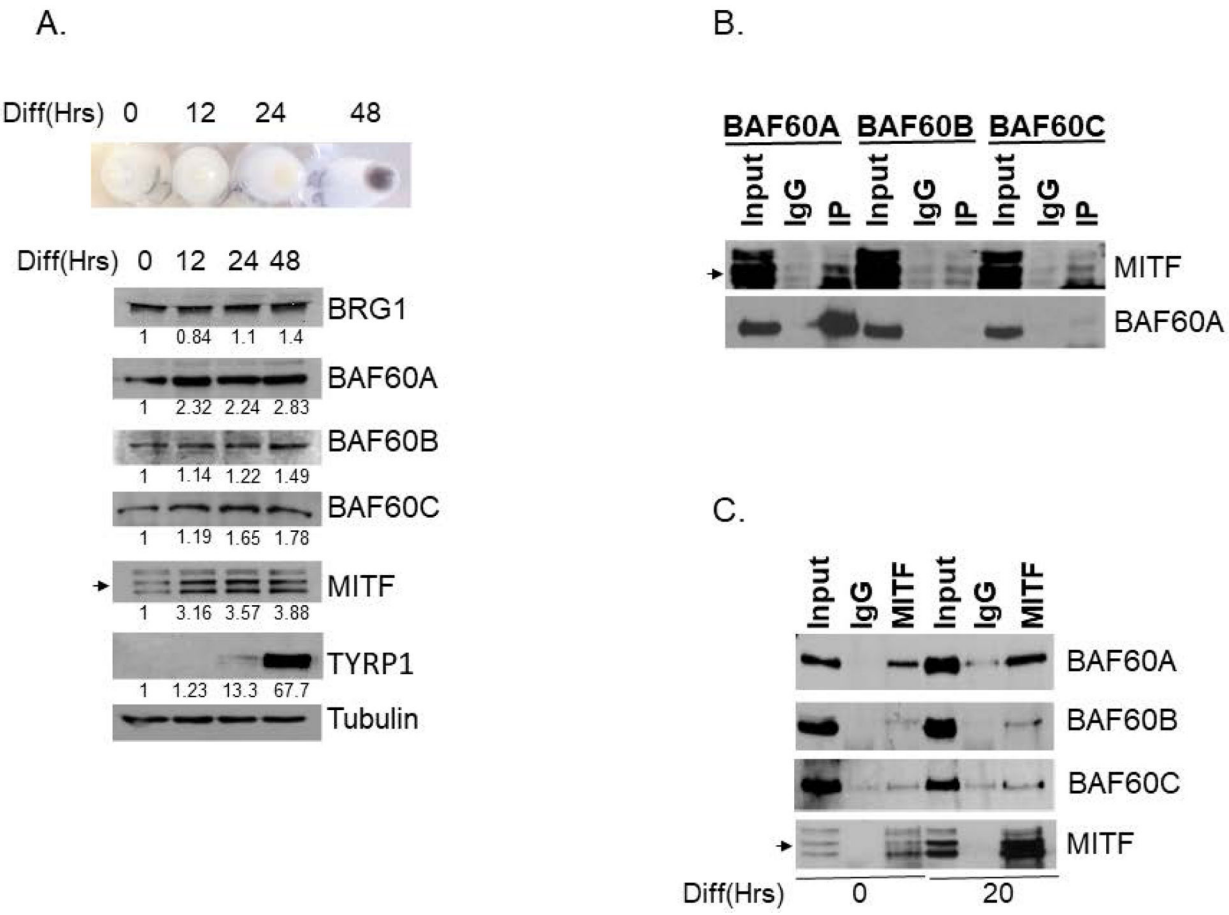

Fig.1. subunit expression and interaction with MITF in Melb-a cells

(A) Top: Undifferentiated (0 Hr) and differentiating Melb-a cells at 12, 24, and 48 Hrs were pelleted and photographed. Bottom: Protein extracts were prepared from differentiating Melb-a cells at each time point and subjected to Western blotting with the indicated antibodies. Tubulin was used as a loading control. Band intensity was determined by Image J. After subtracting background, band intensities were normalized to those of Tubulin at each time point. **(B)** Melb-a cells that had been differentiated for 24 hours were immunoprecipitated with an irrelevant antibody (IgG) or with rabbit antibodies to BAF60A (A301–594), BAF60B (A301–596), or BAF60C (ab50566). Cell extract (Input) or the immunoprecipitated material was run on an SDS-polyacrylamide gel and blotted with mouse antibodies to MITF (ab12039) or BAF60A (611728). (C) Melb-a cells that had been differentiated for 24 hours were immunoprecipitated with an irrelevant antibody (IgG) or with an antibody to MITF. Cell extract (Input) or the immunoprecipitated material was run on an SDS-polyacrylamide gel and blotted with antibodies to BAF60A, BAF60B, or BAF60C or MITF (ab12029). The arrows indicate the unphosphorylated and phosphorylated bands corresponding to MITF-M. The figures are representative of three or more experiments.

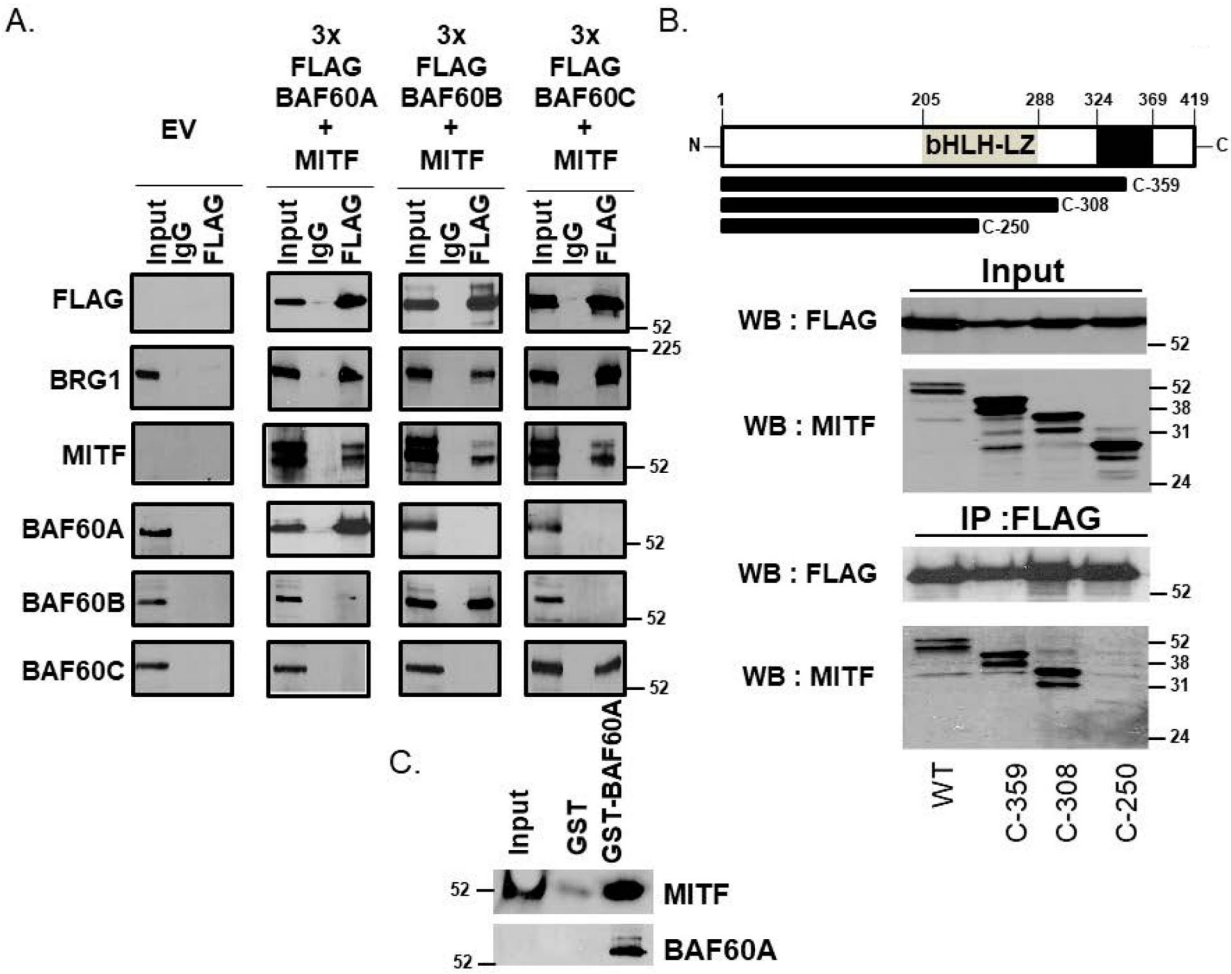

Fig.2. Characterization of MITF interactions with BAF60 subunits

(A) HEK 293T cells were transfected with empty vector or a vector expressing 3X-Flag tagged *BAF60A*, *BAF60B*, or *BAF60C*. Cells were harvested after 48 hours. Whole cell extract was used for immunoprecipitation with IgG as a control or with FLAG antibody tagged Sigma M2 beads. The immunoprecipitated material was run on an SDS – polyacrylamide gel and immunoblotted for FLAG, BRG1, MITF, BAF60A (A301–595), BAF60B (A301–596, BAF60C (ab50566). **(B)** 293T cells were co-transfected with 3X-FLAG *BAF60A* and *Mitf* deletion constructs. Immunoprecipitations were performed with an antibody to FLAG and run on an SDS-polyacrylamide gel. Western blotting was performed with FLAG and MITF antibodies. Top: Schematic showing C terminal deletion constructs relative to the basic helix loop helix-leucine zipper (bHLH-LZ) and the C terminal transactivation domain from 324 to 369 shown in black. Middle: FLAG and MITF expression in cell extract (inputs). Bottom: FLAG and MITF expression detected from FLAG immunoprecipitations. **(C)** BL21 bacterial cells were transformed with either an MITF plasmid or a plasmid expressing GST or GST-BAF60A, cultured, and induced with IPTG for 4 hours. GST or GST-BAF60A was immunoprecipitated with GST-agarose. The beads were then incubated with extract from MITF transformed cells and pulldowns were performed. Inputs are from MITF transformed cells.

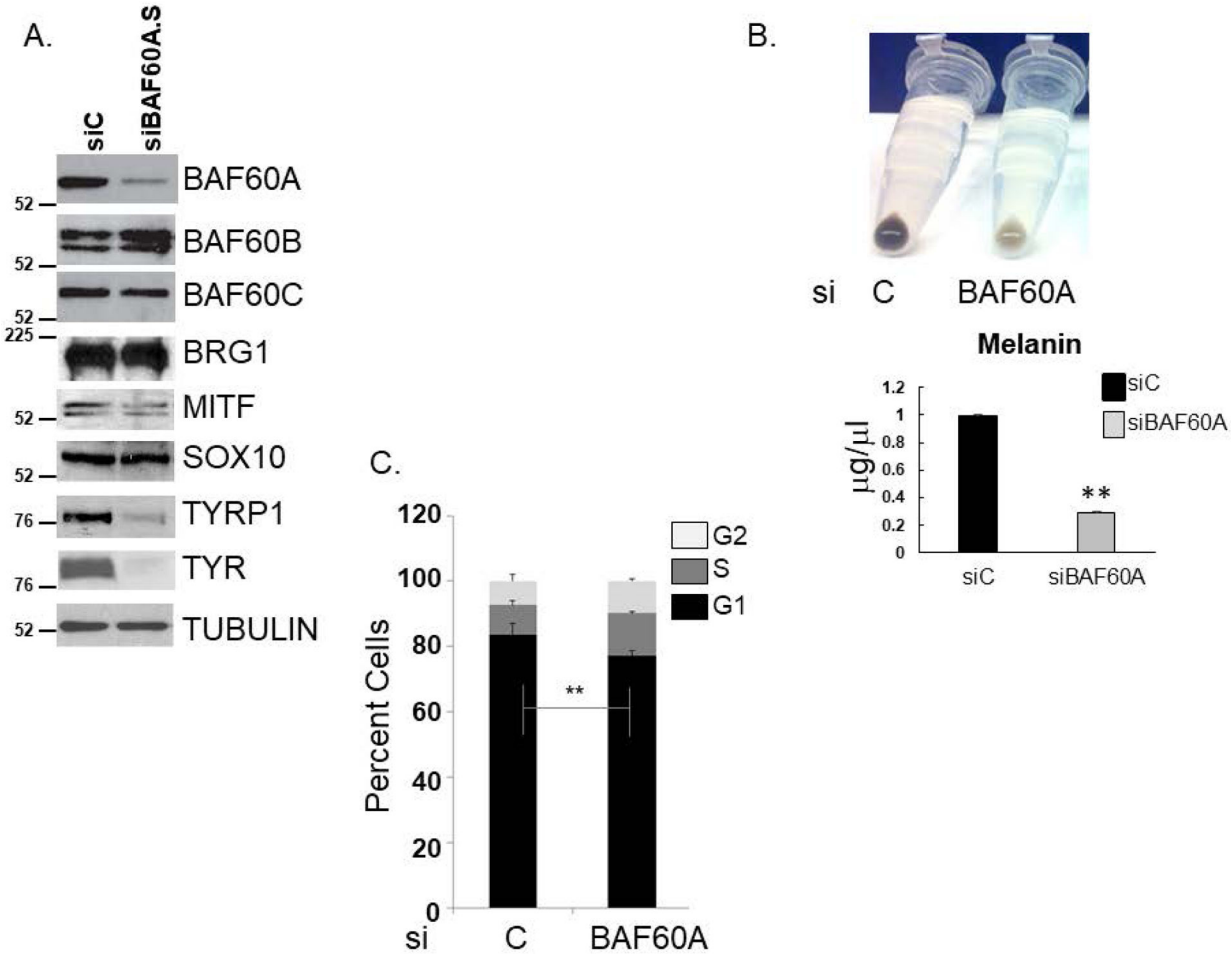

Fig. 3. Effects of BAF60A depletion on melanoblast differentiation and cell cycle profile

(A) Melb-a cells were transfected with control siRNA or a pool of 4 siRNA sequences targeting BAF60A. After 72 hours, growth media was replaced with differentiation media. Protein extracts were prepared from cells that were differentiated for 48 hours and run on an SDS-polyacrylamide gel. Western blotting was performed with the indicated antibodies. Tubulin is shown as a loading control. The figure is representative of three or more experiments. **(B)** Top: A representative picture of cell pellets from differentiated Melb-a cultures that had been transfected control siRNA or a pool of 4 siRNA sequences targeting BAF60A. Bottom: Control and BAF60A depleted Melb-a cells were subjected to the melanin assay. The data are from two experiments performed in triplicate. Statistical significance was calculated by the student's T test (**p<0.01). **(C)** Control and BAF60A depleted Melb-a cells were obtained as in A then stained with propidium iodide and subjected to flow cytometry.

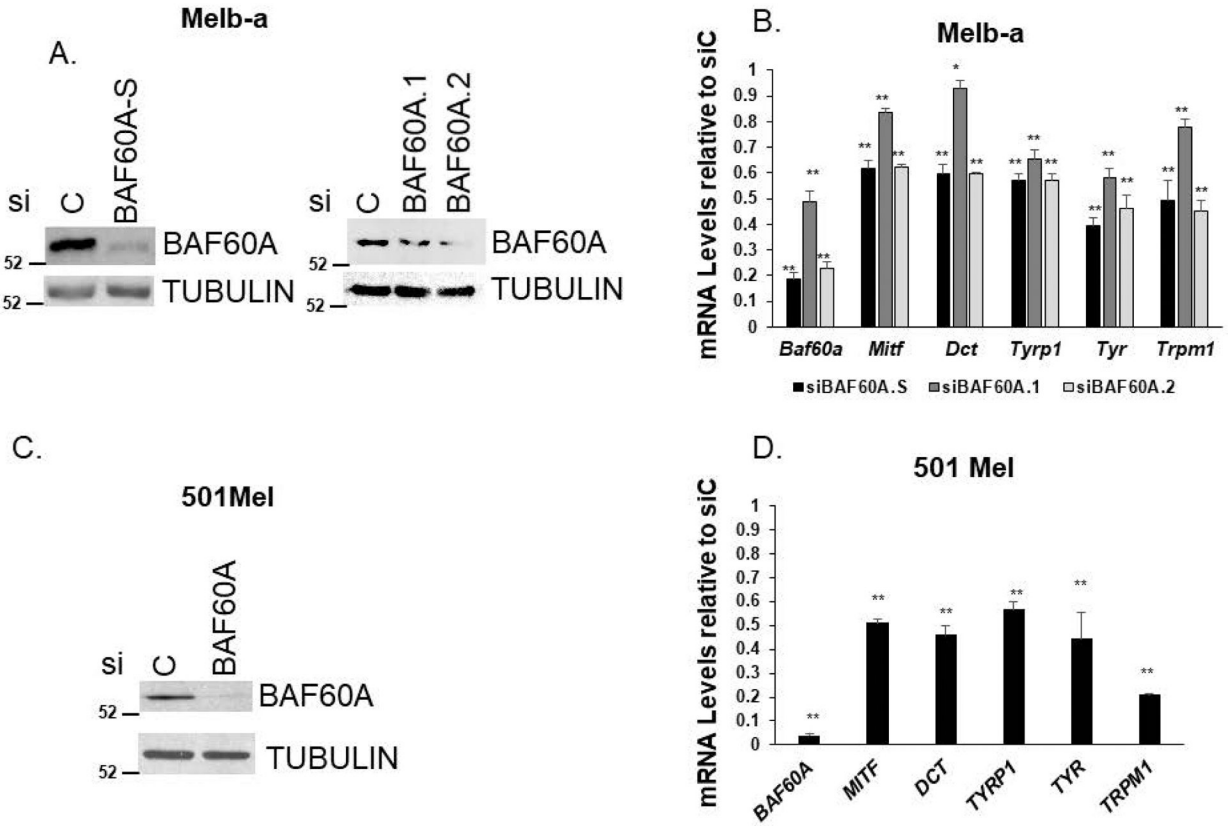

Fig. 4. Effects of BAF60A depletion on MITF and MITF target gene expression in Melb-a cells and 501 Mel melanoma cells

(A) Western blots of Melb-a cells that were transfected with control siRNA, a pool of 4 siRNA sequences (left) or two different siRNA sequences targeting BAF60A (right) as in Fig. 3A. **(B)** RNA was isolated from cells that were transfected with control siRNA or siRNAs targeting BAF60A as in (A), reverse transcribed, and subjected to quantitative PCR with primers to the indicated genes. Relative mRNA levels were calculated by using RPL7 as a control and normalizing to siC values. The data are from two or more experiments performed in triplicate. Statistical significance was calculated by the student's T test (*p<0.05, **p<0.01). **(C)** 501Mel melanoma cells were transfected with control siRNA (siC) or a pool of 4 siRNA sequences targeting BAF60A. Protein extracts were prepared from 501MEL cells harvested 72 hours after transfection and subjected to Western blotting to detect BAF60A depletion. **(D)** RNA was isolated from 501 MEL cells, reverse transcribed and subjected to qRT-PCR as in (C) except that values for each gene were normalized to RPL19. The data are from two experiments performed in triplicate. Statistical significance was calculated by the student's T test (**p<0.01).

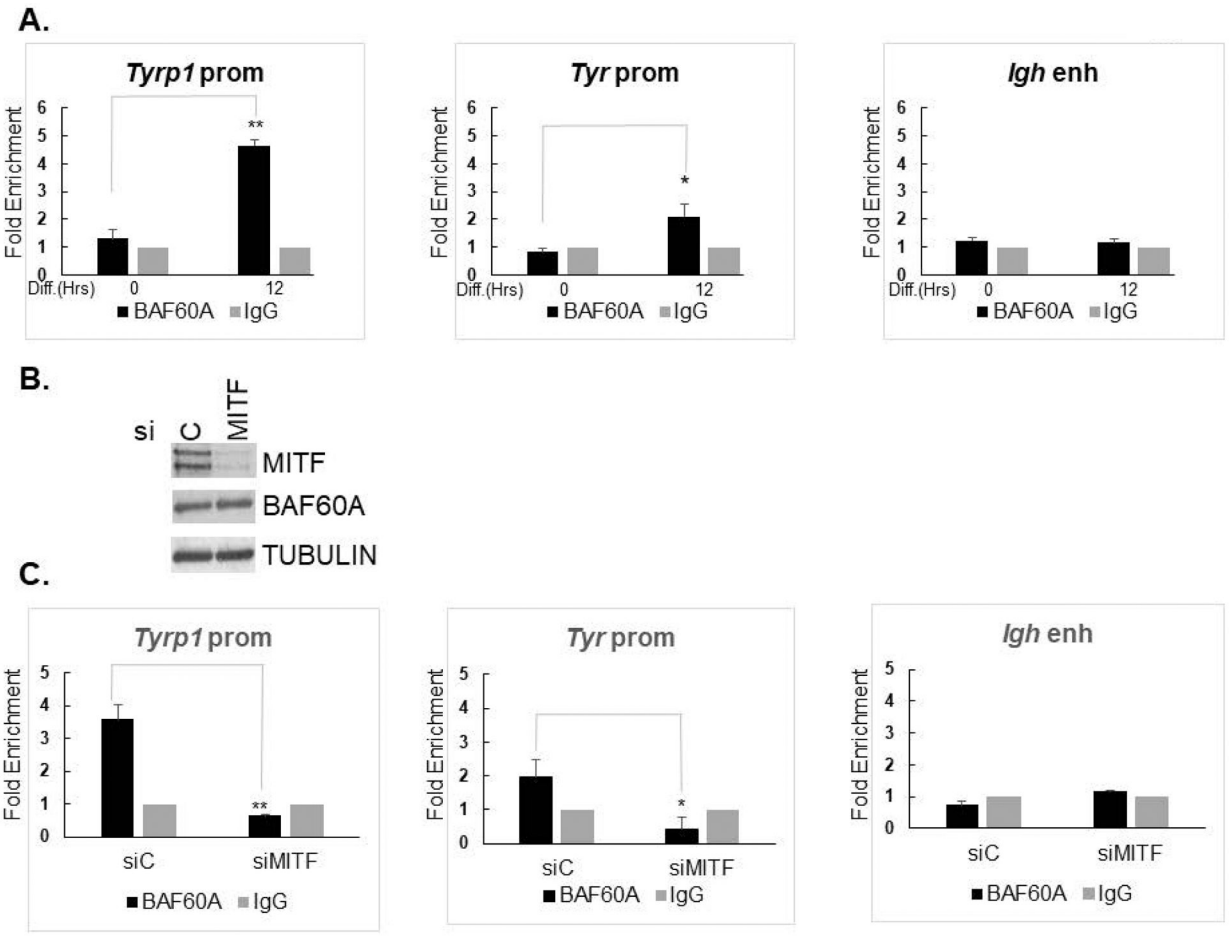

Fig. 5. BAF60A recruitment to MITF target gene promoters

(A) Undifferentiated Melb-a cells or cells that were differentiated for 12 hours were harvested for the ChIP assay. The ChIP assay was performed using an antibody to BAF60A or as a control, IgG. BAF60A enrichment on the *Tyrp1* and *Tyr* promoters was normalized to enrichment of control IgG. Levels of BAF60A enrichment relative to control IgG are shown on the *Tyrp1* and *Tyr* promoters, and as a control the *IgH* enhancer region. **(B)** Melb-a cells were transfected in growth medium with control siRNA or siRNA targeting MITF for 72 hours. Growth medium was replaced with differentiation medium and the cells were cultured for an additional12 hours. Protein extracts were prepared, run on an SDS-polyacrylamide gel and immunoblotted with the indicated antibodies. Tubulin is a loading control. **(C)** Cells were harvested for the ChIP assay and enrichment of BAF60A was quantified as in (A). ChIP data are the averages of three or more experiments. Statistical significance was calculated by the student's T test (**p<0.01).

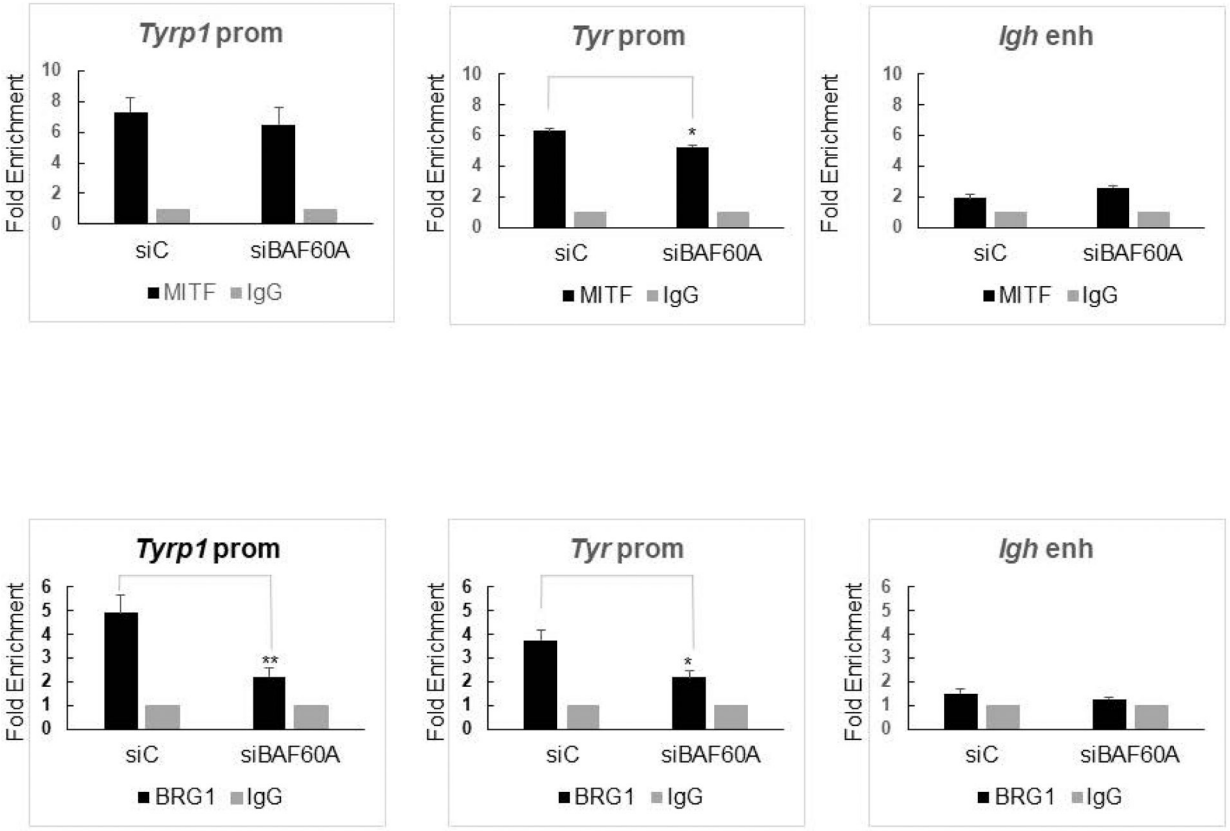

Fig.6. Effects of BAF60A depletion on MITF and BRG1 recruitment to MITF target gene promoters

(A) Melb-a cells were transfected in growth medium with control siRNA or a pool of siRNAs targeting BAF60A for 72 hours. Growth medium was replaced with differentiation medium and the cells were cultured for an additional12 hours. Cells were harvested for the ChIP assay and enrichment of MITF was quantified on the *Tyrp1* and *Tyrp1* promoters and as a control the IgH enhancer as in Fig. 5A. **(B)** ChIPs were performed on Melb-a cells as in (A) except that the antibody was to BRG1. ChIP data are the averages of three or more experiments. Statistical significance was calculated by the student's T test (**p<0.01).

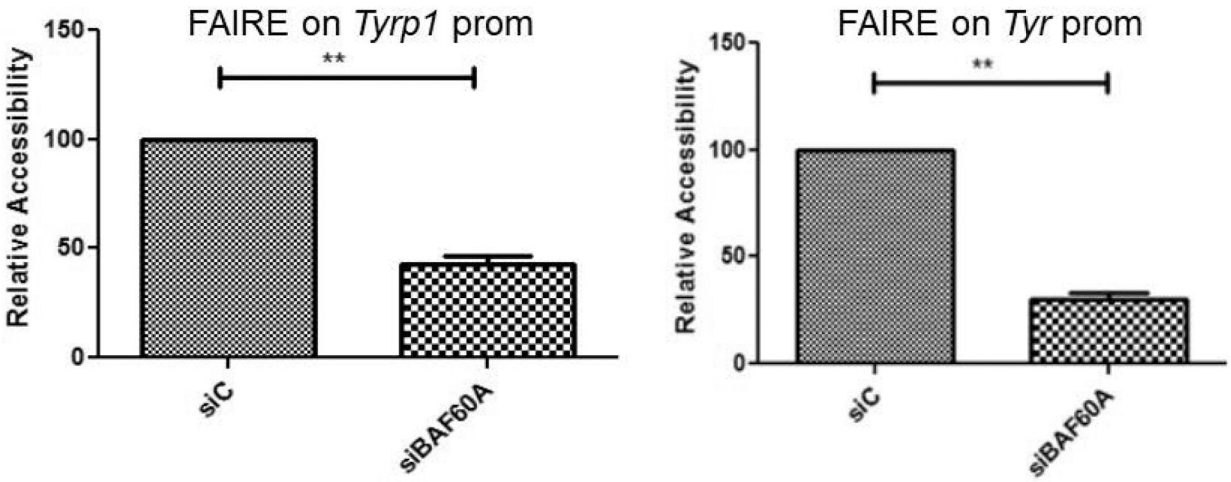

Fig.7. Effects of BAF60A depletion on chromatin accessibility at MITF target promoters

Melb-a cells were transfected in growth medium with control siRNA or a pool of siRNAs targeting BAF60A for 72 hours. Growth medium was replaced with differentiation medium and the cells were cultured for an additional12 hours. Cells were harvested for the FAIRE assay. Chromatin accessibility at the *Tyr* (left) and *Tyrp1* (right) promoters is shown relative to a control *IgH* enhancer region. FAIRE data are the averages of three or more experiments. Statistical significance was calculated by the student's T test (**p<0.01).

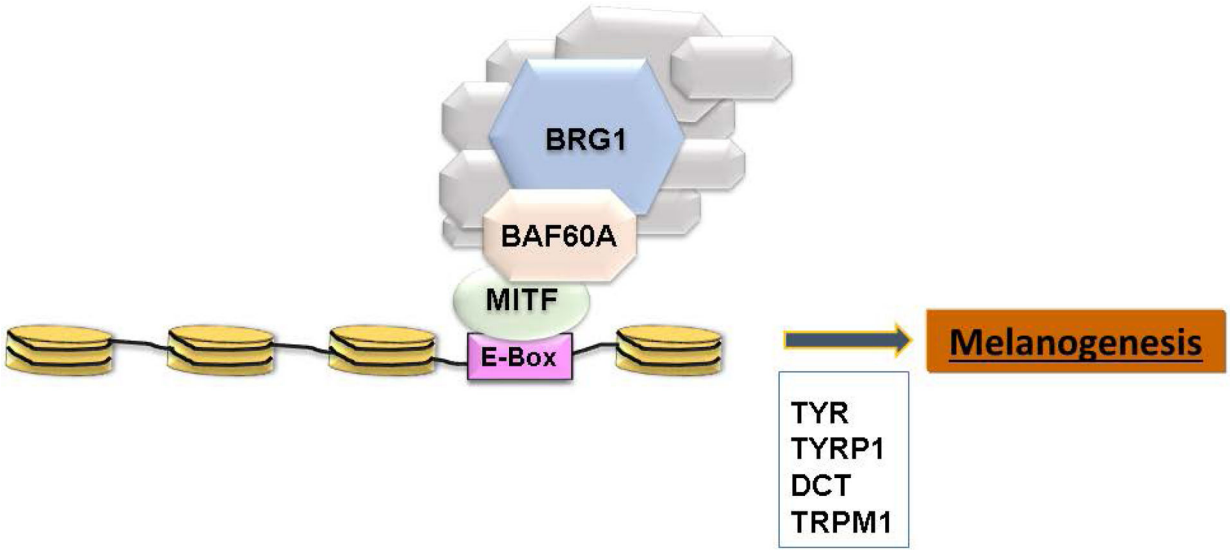

Fig. 8. BAF60A mediates interactions between MITF and BRG1-containing SWI/SNF complexes.

Schematic model depicting BAF60A as a SWI/SNF subunit that mediates interactions between MITF and BRG1 containing SWI/SNF complexes. BAF60A was found to be required for recruitment of BRG1 to promoters of MITF target genes, chromatin remodeling, gene expression, and melanin synthesis.