

## Supplemental Information:

### Plasmids, cloning and retroviral vectors

pGEX 4T1 hBAF60c1, pGEX 4T1 hBAF60c2 were obtained from Auwerx. Threonine 229 of BAF60c was mutated to Alanine to obtain a mutant BAF60c using the quikchange mutagenesis kit (Stratagene) following the manufacturer's indications. Forward primer: 5' GTGGCATCGGGCACCCACGACCC 3'; reverse primer: 5' GGGTCGTGGGTGCCCGATGCCAC 3'.

Retroviral vectors containing the WT or mutant version of BAF60c (pBMNI-IRES-GFP BAF60c WT and pBMNI-IRES-GFP-BAF60c mutant) were obtained by using pGEX 4T1 hBAF60c2 WT or mutant as a template for a PCR with primers that include the whole CDS with restriction sites on both ends and a N-terminal FLAG tag. Forward primer: 5' GCCTCGAGATGGATTACAAGGATGACGACGATAAGAATGGCCGCGGACG 3', reverse 5'GCCTCGAGCTAGGTGTTGCGCACAACCAGCGAC 3'. The amplified products were digested with Xho I and cloned into pBMNI-IRES-GFP retroviral vector (Addgene). Mouse MyoD cDNA was cloned into pcDNA3 vector with N'-terminal FLAG tag. Flag MyoD ΔCt: mouse MyoD (aa 1-217) with truncated C'-terminus was cloned into pcDNA3 vector with N'terminal FLAG tag. For xpBAF60c, mouse BAF60c was cloned into pcDNA3.1c vector (Invitrogen)

A retroviral vector containing a small hairpin RNA (shBAF60c) was obtained by cloning the following annealed primers into BglII and XhoI sites of the pSUPER Puro (Oligoengine) following the manufacturer's protocol. Forward primer: 5'gatcCCCAACAGATGGGTTCCAAGTGTTC AAGAGACACTTGG AACCCATCTGT TTTTTTC 3', Reverse: 5' tcgaGAAAAAACAGATGGGTTCCAAGTGTCTCTTGAACACTTGG AACCCATCT GTTGGG 3'.

### Expression and purifications of recombinant transcription factors

Sf-9 cells were infected with baculovirus encoding Flag-tagged MyoD (monomer), MyoD~MyoD (forced homodimer), MyoD~E12 (forced heterodimer), MEF2D, p300, and pCAF as previously described (Dilworth *et al*, 2004; Rampalli *et al*, 2007). Flag-tagged proteins were purified from whole cell lysates under stringent conditions (Dilworth *et al*, 2004), and eluted with 3X Flag peptide (Sigma). SWI/SNF complex was purified from Hela cells expressing Flag-tagged In1 as previously described (Sif *et al*, 1998). The ability of the purified transcription factors to bind either E-boxes or MEF2 binding elements were confirmed using gel-shift analysis while p300 and pCAF activity were confirmed using *in vitro* histone acetyltransferase assays (data not shown). His-tagged Phospho-p38 (Khokhlatchev *et al*, 1997) and GST-BAF60c (wt or T229A mutant) were purified from *E.coli*.

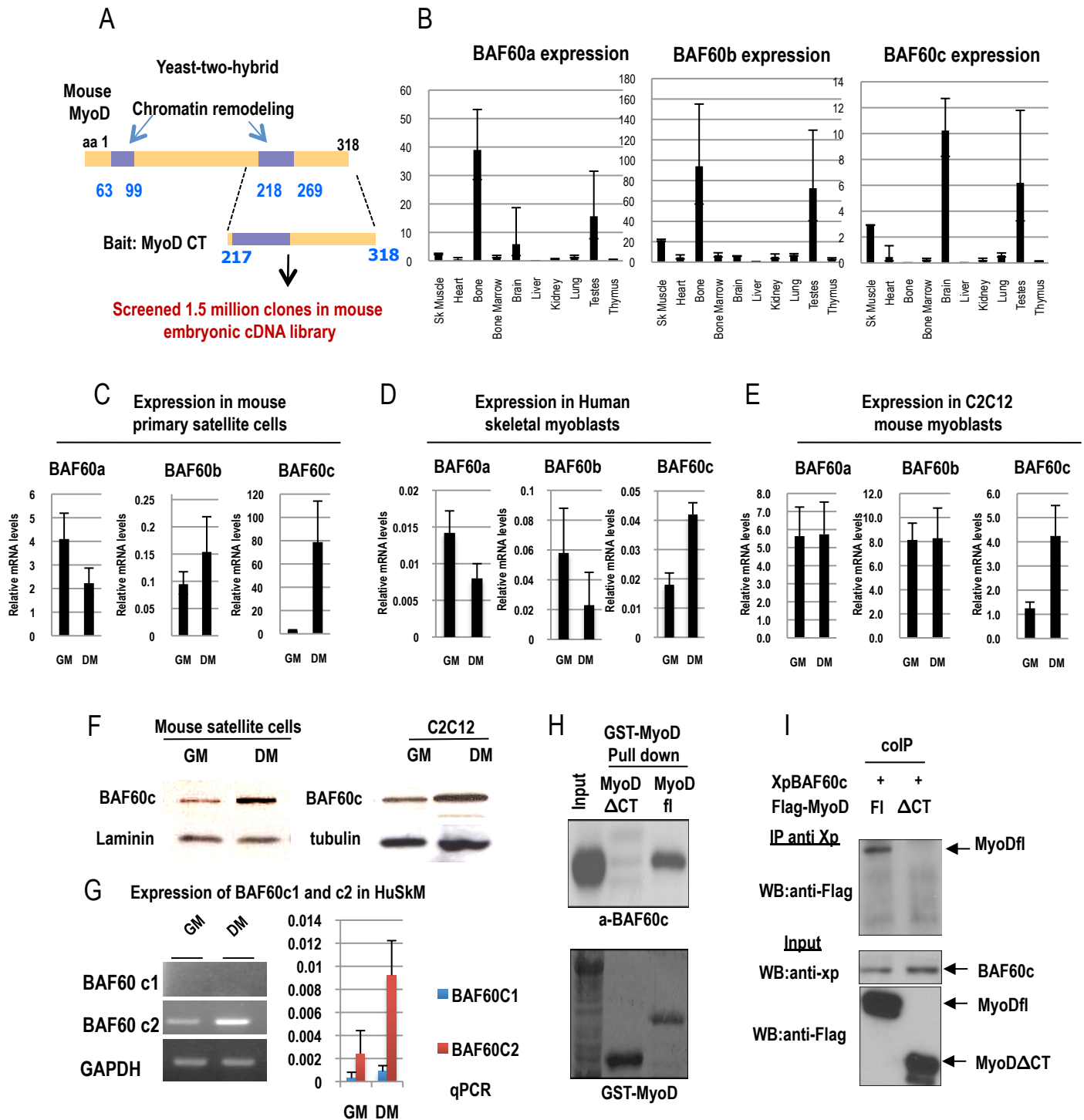
**Antibodies used:**

**PLA:** MyoD 1:200, (BD pharmingen, 554130); Brg1 1:1000 (Santa Cruz, sc-17796X); rabbit Flag 1:1000 (Sigma ); mouse monoclonal Flag 1:1000 (Sigma, F1804) ; MyoD 1:200 (Santa Cruz, SC-760) and phosphothreonine Proline-directed 1:1000 (Cell Signaling 9391S)).

**Western Blot:** BAF60c (Dr. Auwerx, Debril *et al*, 2004) and custom made anti-BAF60c antibody against the epitope SFSRDPKGYVQDLLRSQSRDLK (Openbiosystems), MyHC (MF20, DSHB), myogenin (DSHB), MyoD (Santa Cruz, SC-760, BD pharmingen, 554130), b-tubulin (Santa Cruz, sc-9104), Lamin A/C (Cell Signaling, 2032), Brg1 (Santa Cruz, sc-17796X), Brm (Abcam, Ab15597), Ini1 (Dr. Imbalzano), anti Flag-HRP (Sigma, A8592), anti-Flag (Sigma A rabbit, F1804 mouse monoclonal), Anti Flag M2 agarose (Sigma, A2220), phospho threonine proline-directed (Cell Signaling 9391S), acetyl histone H3 K9 (Millipore, #CS200570), Anti-XP (Santa Cruz), anti-phospho p38 (Santa Cruz, SC-535) and anti-total p38 alpha (Simone *et al*, 2004).

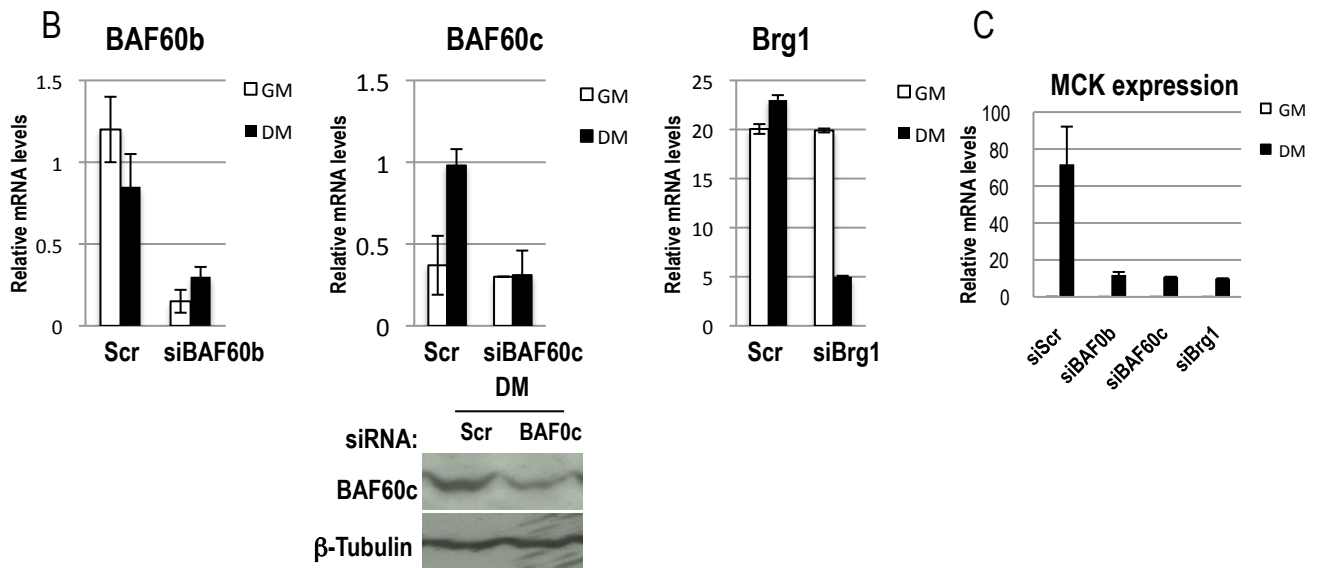
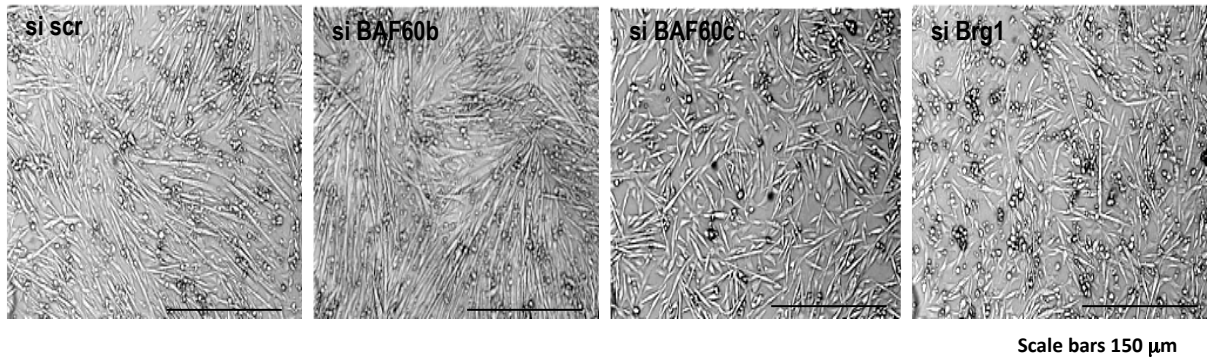
**CoIP:** anti-MyoD (Santa Cruz, SC-760), anti Brg1 (Santa Cruz, sc-17796X) and custom made anti-BAF60c antibody against the epitope SFSRDPKGYVQDLLRSQSRDLK (Openbiosystems) and revealed by western blot with the same antibodies.

**ChIP:** Anti diacetyl (K9/18) histone H3 (Upstate, 07-593), anti-trimethyl K4 Histone H3 (Millipore 17-614), anti-MyoD (Santa Cruz, sc-760) anti-Brg1 (Santa Cruz, sc-17796X), anti-Ini1 (from Imbalzano) custom made chip-validated anti-BAF60c antibody against the epitope SFSRDPKGYVQDLLRSQSRDLK (Openbiosystems).

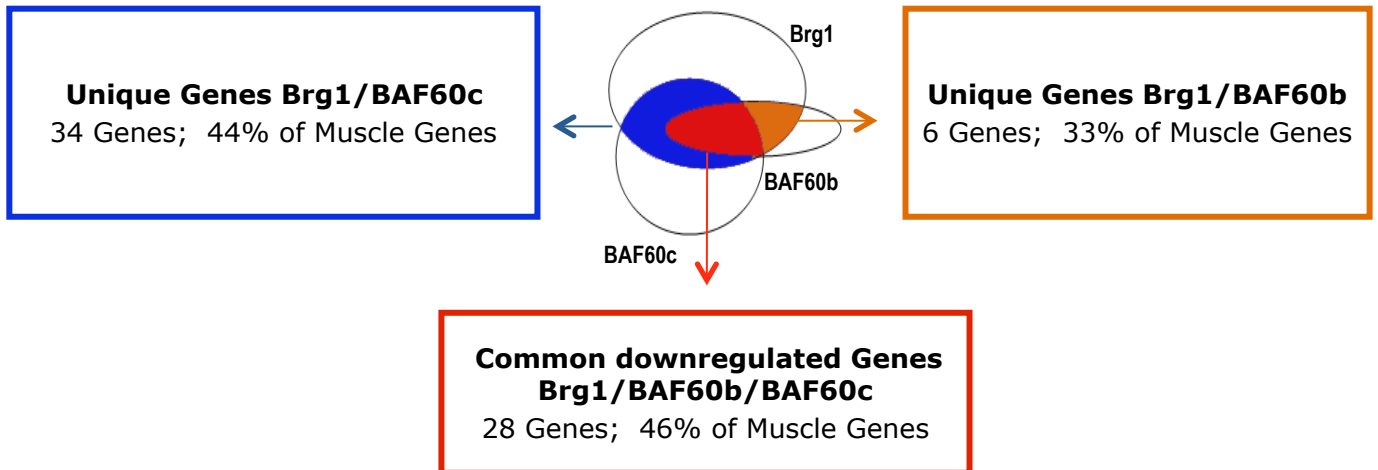


**Supplementary Figure 1. Expression levels of BAF60 variants in skeletal muscle cells and physical interactions between BAF60c and MyoD.** (A) Scheme of the two hybrid system using c-terminal MyoD as a bait. (B) Relative RNA expression of BAF60 sub-units in different mouse organs and tissues. (C-E) RNA expression levels of BAF60 sub-units in undifferentiated (GM – growth medium) or differentiating (DM – differentiation medium for 24 hours) primary mouse satellite cells (C), primary human skeletal myoblasts (D) and mouse C2C12 cells (E), by quantitative RT-PCR (normalized with GAPDH). (F) Protein expression levels of BAF60c in undifferentiated (GM) or differentiating (DM) mouse C2C12 cells (left panel) and primary mouse satellite cells (right panel), by western blot. (G) Semi-quantitative and quantitative RNA levels of human BAF60c1 and c2 variants in primary human skeletal myoblasts (HuSkM). (H) Pull-down assay using GST-MyoD full length (fl) and C-terminal mutant ( $\Delta$ CT) incubated with extracts from COS7 cells previously transfected with XP-tagged BAF60c. The western blot was revealed by anti-XP antibody. (I) Co-immunoprecipitation of transfected XpBAF60c and full length or C-terminal mutant Flag-MyoD in COS7 cells.

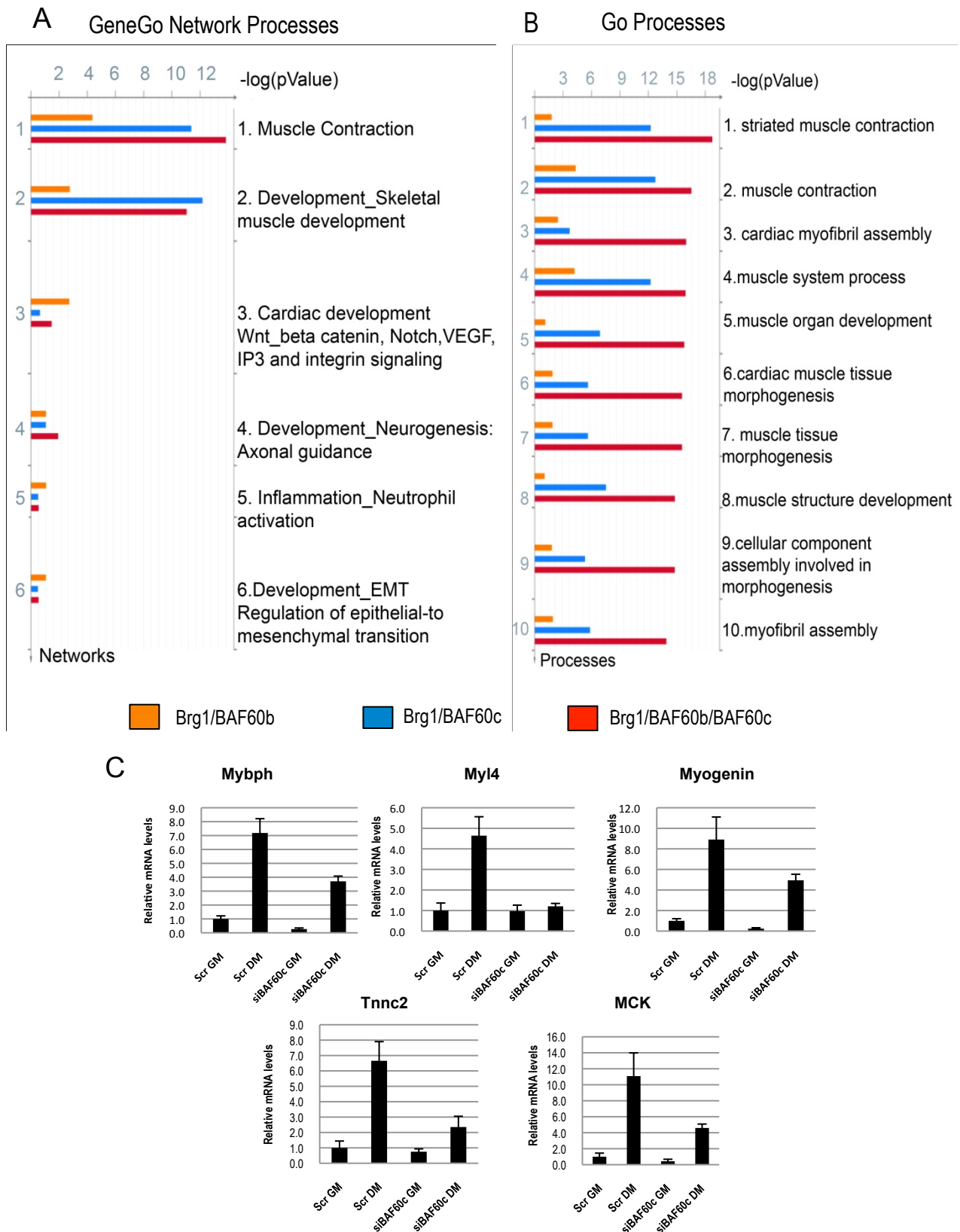
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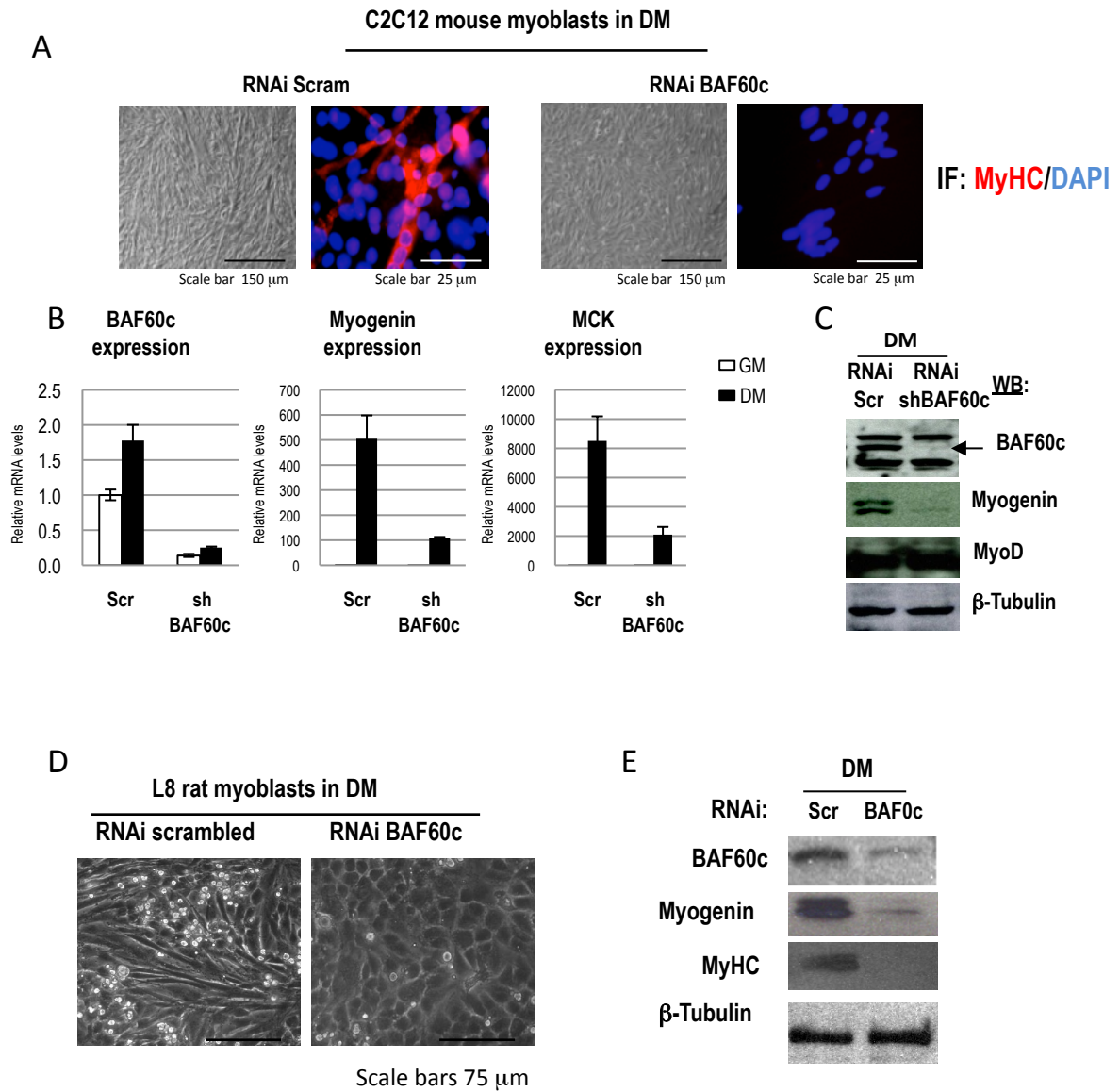
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**Supplementary Figure 2. BAF60c/Brg1-based SWI/SNF controls the expression of MyoD target genes.** (A) Effect on cell morphology of siRNA-mediated knockdown of BAF60b, BAF60c or Brg1 (with scramble siRNA, as control) in C2C12 induced to differentiate (DM for 24 hours). (B) Expression of BAF60b, BAF60c and Brg1 was monitored by qPCR in C2C12 induced (DM) or not (GM) to differentiate after siRNA-mediated knockdown of BAF60b, BAF60c or Brg1 (with scramble siRNA, as control). BAF60c protein levels are measured by western blot (see panel below). (C) Expression of MCK was monitored by qPCR in C2C12 induced (DM) to differentiate after siRNA-mediated knockdown of BAF60b, BAF60c or Brg1 (with scramble siRNA, as control). (D) Class of genes downregulated by the siRNA of Brg1, BAF60b and BAF60c and relative percentage of muscle genes.

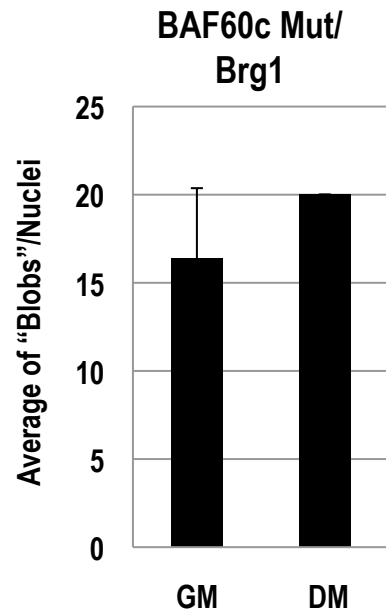


**Supplementary Figure 3. GeneGo classification for process networks and GO process. (A, B)** Categories of genes that are downregulated by the depletion of Brg1, BAF60b or BAF60c in C2C12 myoblasts induced to differentiate. Red label indicates categories of genes that are downregulated in cells depleted by each of these SWI/SNF components. Blue label indicates categories of genes that are downregulated in cells depleted either Brg1 or BAF60c. Orange indicates categories of genes that are found downregulated in cells depleted by either Brg1 or BAF60b. **(C)** qRT-PCRs validation of the microarray experiment for genes downregulated by the absence of BAF60c expression. Normalization is made against GAPDH mRNA levels.

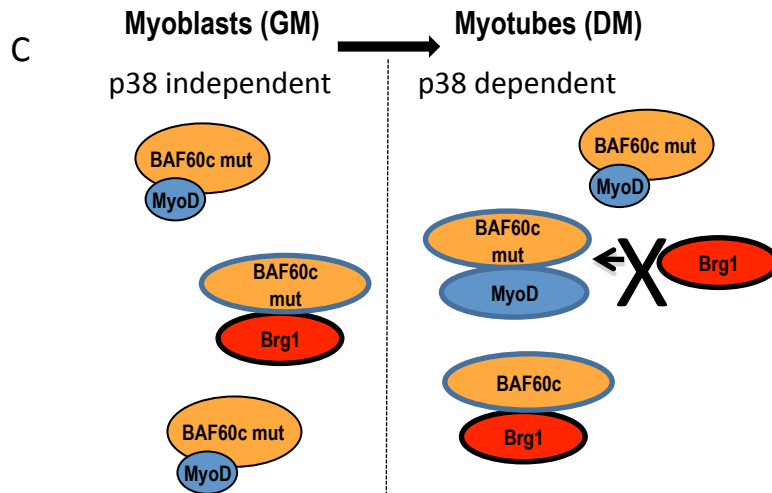
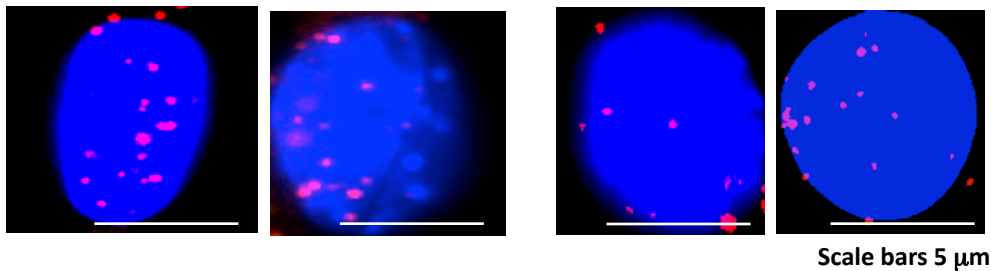
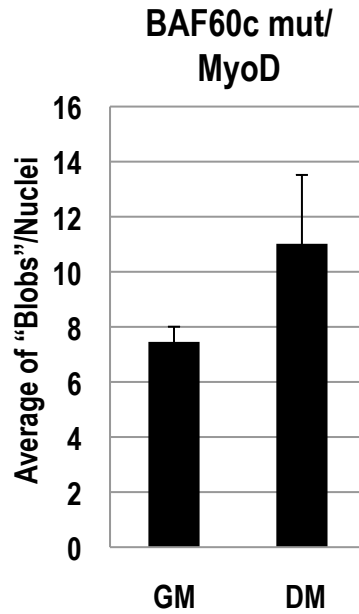


**Supplementary Figure 4. BAF60c is essential for myogenic differentiation.** (A-C) Knock-down of BAF60c by shRNA interference in mouse C2C12 myoblasts blocks the formation of myosin heavy chain (MyHC)-positive myotubes (A) and prevents the expression of differentiation genes (B and C). (D-E) Knock down of BAF60c expression by RNAi in rat L8 myoblasts blocks the formation of myotubes (D) and prevents the expression of differentiation genes (E).

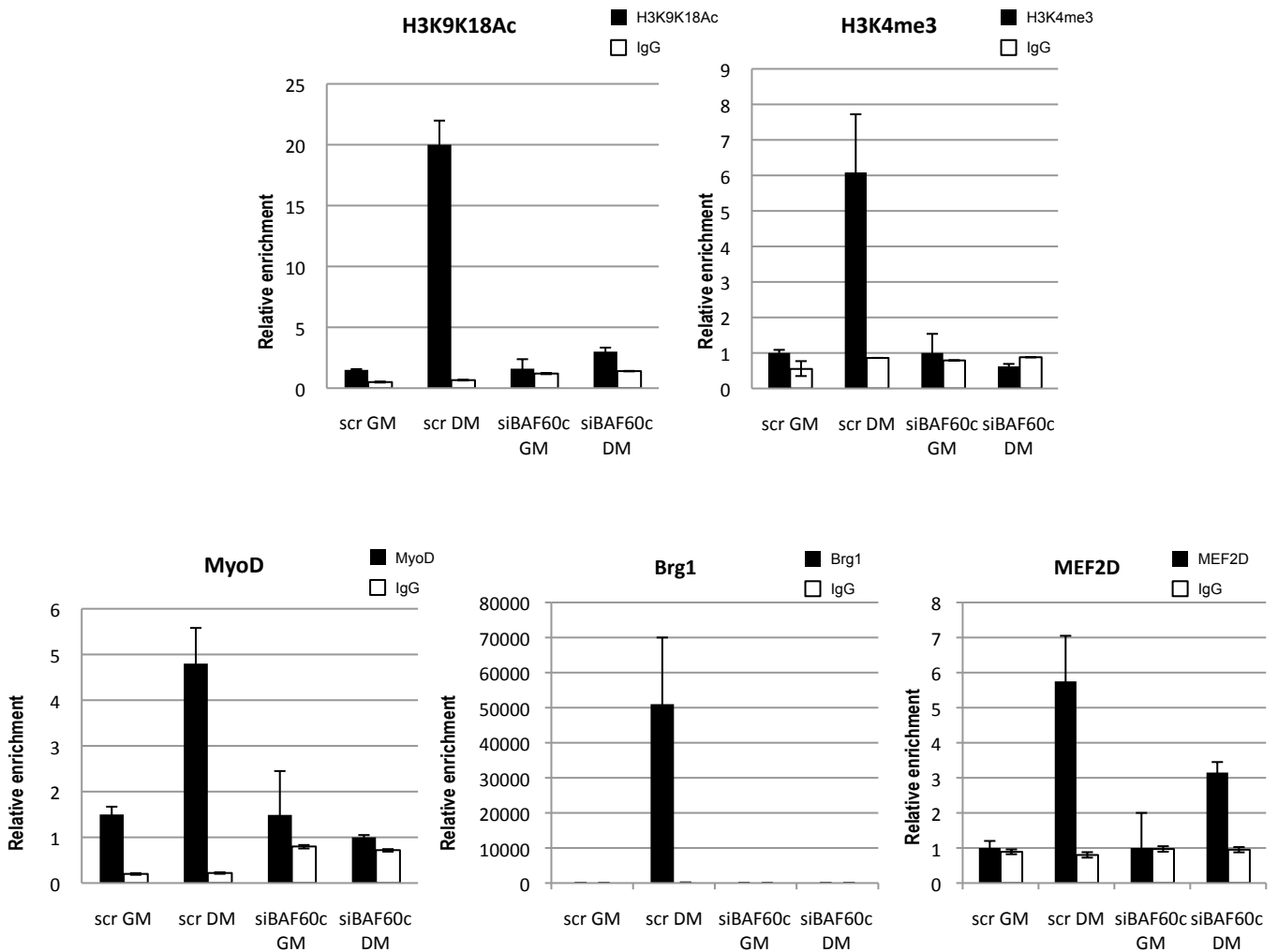
A



B



**Supplementary Figure 5. Phosphorylation resistant BAF60c mutant impairs binding with Brg1 but not with MyoD.** (A-B) Proximity Ligation Assay (PLA) was used to monitor nuclear “in situ” interactions between MyoD and Brg1 with the mutant Flag-BAF60c in myoblasts (GM) and myotubes (DM). Each fluorescent dot, “blob”, represents the co-localization of the indicated proteins. Localization and quantification of the “blobs” was performed with the BlobFinder software (Allalou and Wälby 2009). The average of blobs/nuclei in the graphic corresponds to the quantification of several images from 3 different experiments. (C) Schematic illustration of the p38-independent binding of BAF60c with MyoD and Brg1 in myoblasts. The p38-dependent binding of BAF60c-MyoD with Brg1 is impaired by the BAF60c phosphorylation-resistant mutant.



**Supplementary Figure 6. BAF60c knockdown impairs the recruitment of the myogenic transcriptionosome on myogenin promoter.** ChIP analysis of myogenin promoter for the presence of transcriptional activatory (H3K9K18Ac, H3K4me3) marks and for the recruitment of MyoD, Brg1 and MEF2D in C2C12 undifferentiated (GM) or differentiated (DM) myoblasts transfected with a scrambled RNAi (scr) or BAF60c RNAi (si BAF60c).