

Differential contributions of mSWI/SNF chromatin remodeler sub-families to myoblast differentiation

Teresita Padilla-Benavides^{1*#}, Monserrat Olea-Flores^{1,2#}, Tapan Sharma², Sabriya A. Syed^{2&}, Hanna Witwicka^{2%}, Miriam D. Zuñiga-Eulogio^{1,3}, Kexin Zhang¹, Napoleon Navarro-Tito³ and Anthony N. Imbalzano^{2*}

¹Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT, 06459, USA

²Department of Biochemistry and Molecular Biotechnology, University of Massachusetts Chan Medical School, Worcester, MA, 01605, USA

³Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Guerrero, Mexico

*Correspondence: T.P.-B. tpadillabena@wesleyan.edu and A.N.I. Anthony.Imbalzano@umassmed.edu

The authors contributed equally to this manuscript

& Present address: The Jackson Laboratory for Genomic Medicine, Farmington, CT 06032, USA

% Present address: Charles River Laboratories, Shrewsbury MA, 01545, USA

Emails and ORCID:

T.P.-B. tpadillabena@wesleyan.edu 0000-0002-4624-0822

M.O.-F. Monserrat.OleaFlores@umassmed.edu 0000-0003-1813-9062

S.A.S. sabriya.syed@jax.org 0000-0003-3776-3581

T.S. tapan.sharma@umassmed.edu 0000-0003-2495-2236

M.D.Z.-E. miriamzuniga@uagro.mx 0000-0003-2904-8350

N.N.-T. nnavarro@uagro.mx 0000-0003-4911-0545

H.W. hanna.witwicka@crl.com

K.Z. kzhang@wesleyan.edu 0000-0003-4504-0017

A.N.I. Anthony.Imbalzano@umassmed.edu 0000-0003-0744-3413

Figure S1

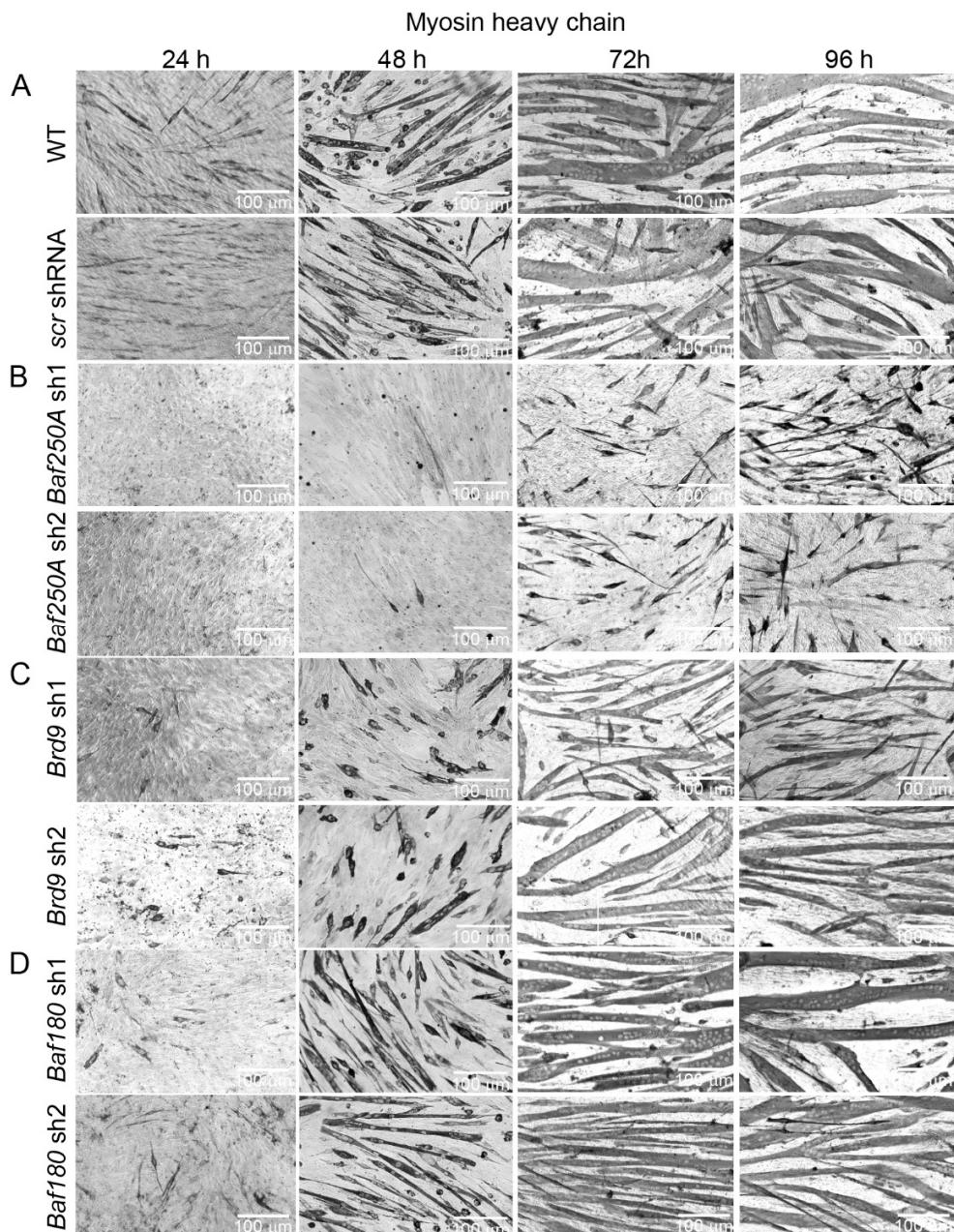


Figure S1. *Baf250A* knockdown inhibited the differentiation of C2C12 cells.

Representative light micrographs of differentiating (**A**) wild type (WT) C2C12 myoblasts and cells transduced with *scr* shRNA (**B**) transduced with *Baf250A* shRNAs, (**C**) transduced with *Brd9* shRNAs, or (**D**) transduced with *Baf180* shRNAs. Cells at 24, 48, 72 and 96 h of differentiation were immunostained for myosin heavy chain. Bars = 100 μ m.

Figure S2

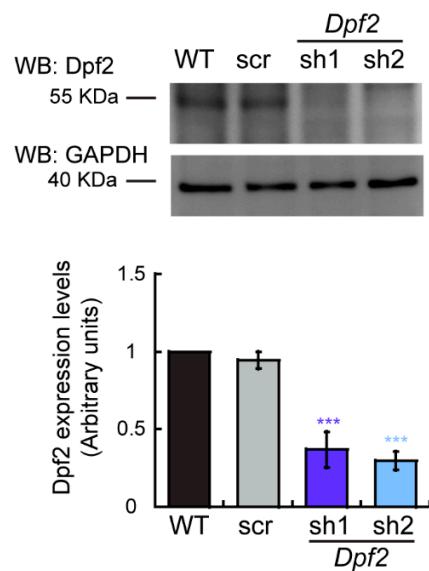


Figure S2. Expression levels of Dpf2 in differentiating wildtype (WT) C2C12 myoblasts. Representative immunoblots (top) and quantification of Dpf2 levels in myoblasts differentiating for 48 h. Immunoblots against GAPDH were used as loading controls. Samples were compared to the wild type sample, the value of which was set at 1.0. Data are the mean \pm SE for three independent experiments. ***P < 0.001.

Figure S3

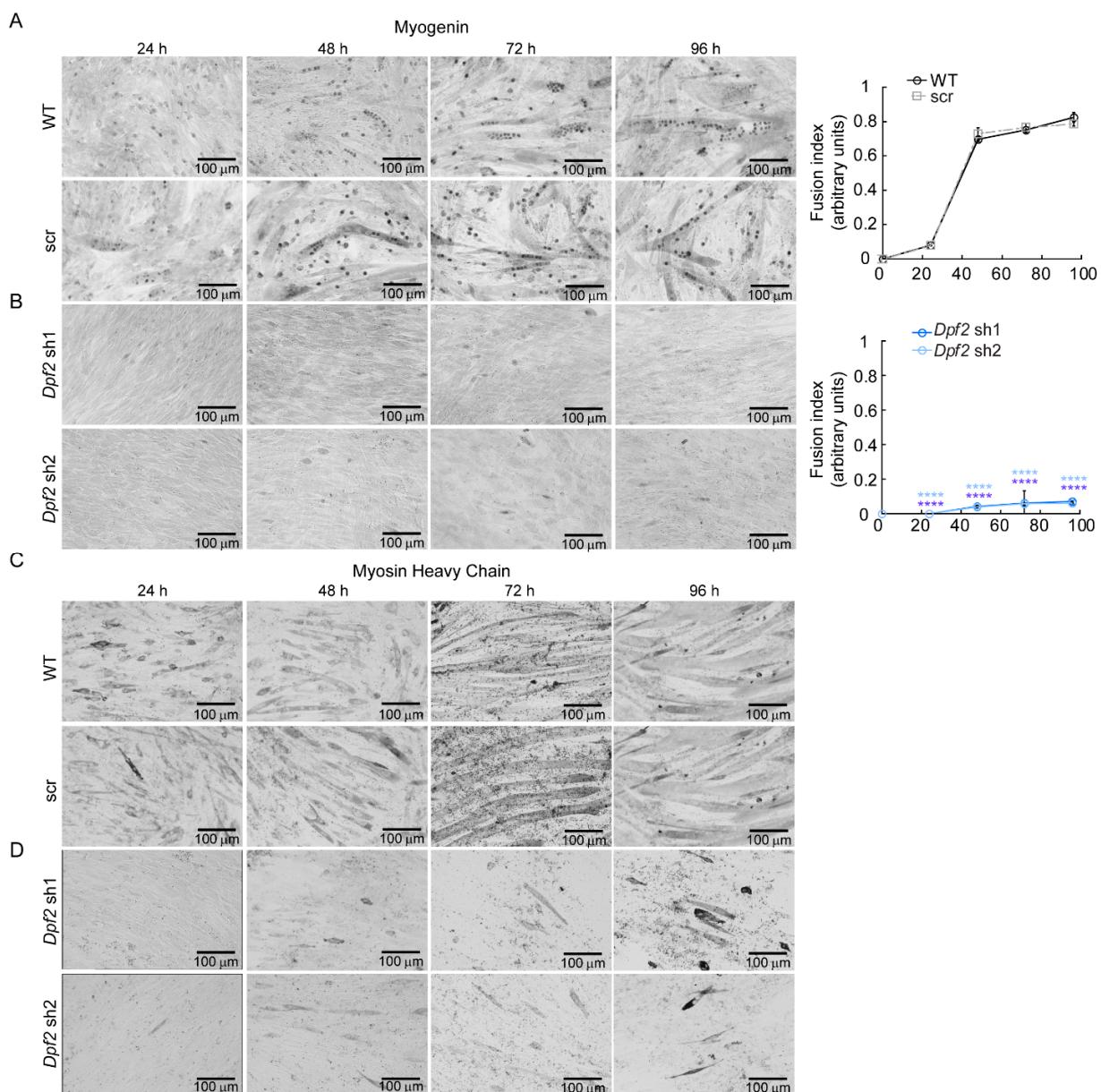


Figure S3. Dpf2 knockdown inhibited the differentiation of C2C12 cells. Representative light micrographs and fusion index of differentiating (**A**) wild type (WT) C2C12 myoblasts and cells transduced with scr shRNA (**B**) transduced with *Dpf2* shRNAs. Cells at 24, 48, 72 and 96 h of differentiation were immunostained for myogenin and myosin heavy chain. Bars = 100 μ m. **** P < 0.0001.

Table S1. Pearson coefficients for the two replicate samples for each KD RNA-Seq dataset

Sample	Pearson coefficient
<i>scr</i> shRNA differentiation	0.96
<i>Baf250A</i> shRNA differentiation	0.98
<i>Brd9</i> shRNA differentiation	0.965
<i>Baf180</i> shRNA differentiation	0.975

Table S3. Sequences of the shRNAs used in this study

Gene name	Sequence	Catalog number (Sigma)
<i>Baf250</i> sh1	CCGGCTTATAGTATGGCGAGTTAACTCGAGTTAACTCGCCA TACTATAAAGTTTTG	TRCN0000238304
<i>Baf250</i> sh2	CCGGCCTAGGCAGCCTAACTATAATCTCGAGATTAGTTAG GCTGCCTAGGTTTTG	TRCN0000238306
<i>Brd9</i> sh1	CCGGTGGACTTGGCACGTAAAGCTCGAGCTTCATCGT GCCAAAGTCCATTTTG	TRCN0000225737
<i>Brd9</i> sh2	CCGGCACCGAATGGTGTCCAATAAGCTCGAGCTTATTGGAC ACCATTGGTGTGGT	TRCN0000225739
<i>Baf180</i> sh1	CCGGTGTGAAGTTGGTCTAGTTACTCGAGTAAACTAGGAC CAACTCACATTTTG	TRCN0000304680
<i>Baf180</i> sh2	CCGGGTGCAATATCCAGACTATTATCTCGAGATAATAGTCTG GATATTGCACATTTTG	TRCN0000304681
<i>Dpf2</i> sh1	CCTGGTGATTACAGGGTCAAA	TRCN0000084343
<i>Dpf2</i> sh2	GCCTAACAACTACTGTGACTT	TRCN0000084345
<i>scr</i> shRNA pLKO.1-puro non- target shRNA control plasmid DNA	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCT TCATCTTGGTGTGGT	MFCD07785395 SHC002

Table S4. List of primers used in this study

Primer name	5' sequence	3' sequence	Use	Reference
<i>q-Myogenin</i>	CAAGTGTGCACATCTGT TCTAGTCTCT	GTATCATCAGCACAGGAGA CCTGGT	Gene expression	Hernandez-Hernandez et al., 2013 ¹
<i>q-Ckm</i>	CTGTCCGTGGAAGCTCT CAACAGC	TTTGTTGTCGTTGTGCCAG ATGCC	Gene expression	Hernandez-Hernandez et al., 2013 ¹
<i>q- MyHCIIb</i>	TCAATGAGATGGAGATC CAGCTGAAC	GTCCAGGTGCAGCTGTGTG TCCTTC	Gene expression	Hernandez-Hernandez et al., 2013 ¹
<i>q-Cav3</i>	TCAATGAGGACATTGTG AAGGTAGA	CAGTGTAGACAAACAGGCGG T	Gene expression	Witwicka et al., 2019 ²
<i>q-Eef1A1</i>	GGCTTCACTGCTCAGGT GATTATC	ACACATGGGCTTGCCAGGG AC	Gene expression	Hernandez-Hernandez et al., 2013 ¹
<i>Myogenin</i> promoter	ACGCCAACTGCTGGGTG CCA	GAATCACATGTAATCCACTG GA	ChIP qPCR	Hernandez-Hernandez et al., 2013 ¹
<i>Ckm</i> enhancer	GACACCCGAGATGCCTG GTT	GATCCACCAGGGACAGGGT T	ChIP qPCR	Hernandez-Hernandez et al., 2013 ¹
<i>MyHCIIb</i> promoter	CACCCAAGCCGGGAGAA ACAGCC	GAGGAAGGACAGGACAGAG GCACC	ChIP qPCR	Hernandez-Hernandez et al., 2013 ¹
<i>Cav3</i> promoter	CCTAGGTGCTCAGTCC AGTTA	CTGCCACGTAGATCTTGGAA AAT	ChIP qPCR	Witwicka et al., 2019 ²
<i>IgH</i> enhancer	GCCGATCAGAACCCAGAA CACC	TGGTGGGGCTGGACAGAGT GTTTC	ChIP qPCR	Hernandez-Hernandez et al., 2013 ¹

Supplemental references

- 1 Hernandez-Hernandez, J. M., Mallappa, C., Nasipak, B. T., Oesterreich, S. & Imbalzano, A. N. The Scaffold attachment factor b1 (Safb1) regulates myogenic differentiation by facilitating the transition of myogenic gene chromatin from a repressed to an activated state. *Nucleic Acids Res* **41**, 5704-5716, doi:10.1093/nar/gkt285 (2013).
- 2 Witwicka, H. et al. Calcineurin broadly regulates the initiation of skeletal muscle-specific gene expression by binding target promoters and facilitating the interaction of the SWI/SNF chromatin remodeling enzyme. *Mol Cell Biol*, doi:10.1128/MCB.00063-19 (2019).