

Supplemental Information

Homeodomain interacting protein kinase 2-dependent repression of myogenic differentiation is relieved by its caspase-mediated cleavage

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Inventory of Supplemental Information

Two supplemental Tables, one supplemental Figure

Supplemental Table 1

Antibodies, plasmids and reagents

Antibodies

Epitope	Species	Designation	Supplier
HIPK2 amino acids 2-191	monoclonal rat	N6A10	this paper
HIPK2 amino acids 781-1191	monoclonal rat	C1B3	this paper
Flag	mouse monoclonal	M2	Sigma
β -tubulin	mouse monoclonal	tub 2.1	Sigma
MyHC	Rabbit polyclonal	H-300	Santa Cruz
Myogenin	Mouse monoclonal	F5D	Santa Cruz
MEF2	Rabbit polyclonal	C-21	Santa Cruz
HDAC3	Rabbit polyclonal	H-99	Santa Cruz

HDAC4	Rabbit polyclonal	H-92	Santa Cruz
Myc	Mouse monoclonal	9E10	Roche Applied Science
Cy3-coupled secondary antibody		106-165-003	Jackson Immunoresearch
Dylight 405-coupled secondary antibody	Goat Anti-Rabbit	111-475-003	Jackson Immunoresearch
HA	rat monoclonal	3F10	Roche Applied Science
GFP	mouse monoclonal	7.1 and 13.1	Roche Applied Science
Acetyl-lysine	rabbit polyclonal	#9441	Cell signaling Technology
HDAC3	rabbit polyclonal	H99	Santa Cruz Biotechnology

Plasmids

Plasmid	Origin	Citation
pLKO.1-puro HIPK2	MISSION® shRNA vectors, Sigma-Aldrich	Mol Cell. 2012 May 25;46(4):472-83.
pLKO.1-puro scramble	MISSION® shRNA vectors, Sigma-Aldrich	Mol Cell. 2010 Feb 26;37(4):503-15.
290-pHAGE	M. Schmidt-Supprian (Martinsried)	Mol Cell. 2012 May 25;46(4):472-83.
packaging vectors pMDLg/pRRE pRSV-Rev pHCMVG	D. Baltimore (Pasadena)	J Virol. 1998 Nov;72(11):8463-71.
290-pHAGE-Flag-HIPK2	Already Described	Mol Cell. 2012 May 25;46(4):472-83.
Flag-MEF2C	XJ. Yang	Mol Cell Biol. 2005

	(Montreal)	March; 25(6): 2273–2287.
HA-MEF2C	XJ. Yang (Montreal)	Mol Cell Biol. 2005 March; 25(6): 2273–2287.
Flag-HDAC1	A.S. Baldwin (Chapel Hill)	Mol Cell Biol. 2001 Oct;21(20):7065-77.
Flag-HDAC2	A.S. Baldwin (Chapel Hill)	Mol Cell Biol. 2001 Oct;21(20):7065-77.
Flag-HDAC3	P. Gallinari (Rome)	Proc Natl Acad Sci U S A. 2007 October 30; 104(44): 17335–17340.
HA-HDAC3	XJ. Yang (Montreal)	Mol Cell Biol. 2007 February; 27(4): 1280–1295
Flag-HDAC4	P. Gallinari (Rome)	Proc Natl Acad Sci U S A. 2007 October 30; 104(44): 17335–17340.
HA-HDAC4	N. Tsumaki (Kyoto)	Development. 2012 Mar;139(6):1153-63
Myc-HDAC4	M. Kracht (Giessen)	Nucleic Acids Res. 2012 Oct 19, in press
Flag-HDAC5	M. Kracht (Giessen)	Nucleic Acids Res. 2012 Oct 19, in press
Flag-HDAC6	XJ. Yang (Montreal)	Mol Cell. 2007 Jul 20;27(2):197-213.
Flag-HIPK2	already described	Nat Cell Biol. 2002 Jan;4(1):1-10.
Flag-HIPK2 K221A	already described	Nat Cell Biol. 2002 Jan;4(1):1-10.
Flag-HIPK2 DD/AA	already described	EMBO J. 2006 May 3;25(9):1883-94.
Flag-HIPK2 1-916	already described	EMBO J. 2006 May 3;25(9):1883-94.
GFP-HIPK2	already described	Nat Cell Biol. 2002 Jan;4(1):1-10.
Myogenin- Luc	P.L. Puri (Rome)	J Biol Chem. 2000 Nov 24;275(47):36750-7.
HA-HIPK2	already described	EMBO J. 2010 Nov 17;29(22):3750-61.
YFP-CBP	already described	Oncogene. 2012 Feb 2;31(5):611-23.

Calcineurin Δ CAM-AI	E. Muñoz (Córdoba)	J Immunol. 2004 Feb 15;172(4):2341-51
pSUPER- shHDAC3	L DiCroce (Barcelona)	Proc Natl Acad Sci U S A. 2006 Jan 31;103(5):1400-5.
HIPK2 shRNA resistant	Point mutagenesis	This paper

Reagents

Reagent	Supplier
TSA	SIGMA
zVAD-fmk	BD Pharmingen
1- β -D-Arabi- furanosylcytosine (AraC)	CALBIOCHEM
Streptavidin magnetic beads	QIAGEN
λ phosphatase	NEB
Hoechst 33342	SIGMA
Lipofectamine 2000	Invitrogen
Rotifect	Carl Roth
PEI	SIGMA

Primers

Primer name	Sequence
MyLC- forward	AAGATCGAGTTCTCTAAGGAGCA
MyLC- reverse	TCATGGGCAGAACTGTTCAA
Myogenin- forward	GAGACATCCCCCTATTTCTACCA
Myogenin- reverse	GCTCAGTCCGCTCATAGCC
HIPK2- forward	ACACCACTAATGGTAGCAACTCT
HIPK2- reverse	GGGTAAAGGGCACCGCATA
Bio-MEF2- forward	Bio-GCAGCAACAGCTTAGAGG

MEF2- reverse	TCCATCAGGTTCGGAAAA
Bio- Luciferase- forward	Bio-GATCTGAGCTTGGCATTTC
Luciferase- reverse	CAGGAACCAGGGCGTA
Mutagenesis primer to make HIPK2 shRNA resistant- Forward	GGAGCATCCACCCATGATCCAAAACAATGCAAGCGGGGCC
Mutagenesis primer to make HIPK2 shRNA resistant- Reverse	GGCCCCGCTTGCTTGCATTGTTTTGGATCATGGGTGGATGCTCC
MEF2 sites in Myogenin promoter ChIP primer Forward	ATGTAATCCACTGGAAACG
MEF2 sites in Myogenin promoter ChIP primer Reverse	CATCAGGTTCGGAAAAGG

Supplemental Figure 1.

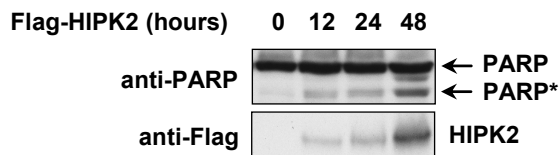


Fig. S1. Cells were transfected to express HIPK2 for the indicated periods. Caspase-mediated cleavage of endogenous PARP1 was revealed with specific antibodies, the cleavage product is shown as PARP*.

Supplemental Table 2

Known HIPK2 interactors as regulators of muscle differentiation and function

HIPK2 interacting protein	Reference
AML1 (RUNX1)	Wang X, Blagden C, Fan J, Nowak SJ, Taniuchi I, Littman DR, Burden SJ. <i>Genes Dev.</i> 2005 Jul 15;19(14):1715-22
NLK	Satoh K, Ohnishi J, Sato A, Takeyama M, Iemura S, Natsume T, Shibuya H. <i>Mol Cell Biol.</i> 2007 Nov;27(21):7623-30.
c-Myb	Kolodziejska KM, Noyan-Ashraf MH, Nagy A, Bacon A, Frampton J, Xin HB, Kotlikoff MI, Husain M. <i>Circ Res.</i> 2008 Mar 14;102(5):554-61.
c-Ski	Kobayashi N, Goto K, Horiguchi K, Nagata M, Kawata M, Miyazawa K, Saitoh M, Miyazono K. <i>Genes Cells.</i> 2007 Mar;12(3):375-85
Smad	Allen DL, Unterman TG. <i>Am J Physiol Cell Physiol.</i> 2007 Jan;292(1):C188-99
Mdm2	Guo CS, Degrin C, Fiddler TA, Stauffer D, Thayer MJ. <i>J Biol Chem.</i> 2003 Jun 20;278(25):22615-22.
PCAF	Sartorelli V, Puri PL, Hamamori Y, Ogryzko V, Chung G, Nakatani Y, Wang JY, Keddes L. <i>Mol Cell.</i> 1999 Nov;4(5):725-34
p53	Soddu S, Blandino G, Scardigli R, Coen S, Marchetti A, Rizzo MG, Bossi G, Cimino L, Crescenzi M, Sacchi A. <i>J Cell Biol.</i> 1996 Jul;134(1):193-204
CBP	Roth JF, Shikama N, Henzen C, Desbaillets I, Lutz W, Marino S, Wittwer J, Schorle H, Gassmann M, Eckner R. <i>EMBO J.</i> 2003 Oct 1;22(19):5186-96
p300	Puri PL, Avantaggiati ML, Balsano C, Sang N, Graessmann A, Giordano A, Levrero M. <i>EMBO J.</i> 1997 Jan 15;16(2):369-83
Daxx	Gupta A, Hou R, Liu L, Hiroyasu S, Hadix JA, Huggins GS, Sibinga NE. <i>J Cell Biochem.</i> 2009 Jun 1;107(3):438-47
JNK	Khurana A, Dey CS. <i>J Muscle Res Cell Motil.</i> 2004;25(8):645-55
CtBP	Zhang CL, McKinsey TA, Lu JR, Olson EN. <i>J Biol Chem.</i> 2001 Jan 5;276(1):35-9.
Groucho	Miller RR, Okkema PG. <i>PLoS Genet.</i> 2011 Aug;7(8):e1002210.
mSin3	van Oevelen C, Bowman C, Pellegrino J, Asp P, Cheng J, Parisi F, Micsinai M, Kluger Y, Chu A, Blais A, David G, Dynlacht BD. <i>Mol Cell Biol.</i> 2010 Dec;30(24):5686-97
HDAC3	Grégoire S, Xiao L, Nie J, Zhang X, Xu M, Li J, Wong J, Seto E, Yang XJ. <i>Mol Cell Biol.</i> 2007 Feb;27(4):1280-95
Nkx	Fu Y, Yan W, Mohun TJ, Evans SM. <i>Development.</i> 1998 Nov;125(22):4439-49