Histone deacetylase 6 regulates cytokinesis and erythrocyte enucleation through deacetylation of formin protein mDia2

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Supplementary Methods and Figures

Methods:

Cells and DNA constructs

MEL cell and NIH 3T3 cells were obtained and cultured as described (1). Retroviral vector pSuper and pSuper HDAC6 shRNA were previously described (2). MSCV-GFP and MSCV-GFP-mDia2 shRNA were described (3). pCR-Blunt-mDia2 plasmid containing mouse mDia2 cDNA was a kind gift from S. Narumiya (Kyoto University, Kyoto, Japan) (4). The shRNA resistant mutant plasmids of pCR-Blunt-mDia2 WT, pCR-Blunt-mDia2K970R, and pCR-Blunt-mDia2K970Q were generated using site directed mutagenesis according to the manufacturer's protocol (Agilent Technologies). The presence of directed mutations was confirmed by Sanger sequencing. The Flag tagged mDia2 and mutants were subsequently cloned into the MICD4 viral vector (5).

Immunostaining: Fetal liver cells were plated in fibronectin-coated 8-well chamber vessels (Corning) and were fixed with 4% paraformaldehyde and 0.4 M sucrose in PBS. Cells were subsequently incubated with 3% control serum in PBS at 4°C overnight and then incubated at room temperature for 2 h with the following primary antibodies: anti-mDia2 (1:200) (ECM Biosciences), anti-HDAC6 (1:200) (Millipore) or goat anti-HDAC6 (1:200) (Santa Cruz). The cells were then incubated with appropriate secondary antibodies coupled to Alexa Fluor 488 (1:1000), or Alexa Fluor 594 (1:1000), and/or Texas-Red phalloidin (1:1000) (Invitrogen). The samples were mounted in UltraCruz mounting medium DAPI (Santa Cruz) on glass slides.

Immunoprecipitation and western blot analysis

The immunoprecipitation and Western blot assays were performed as previously described (6) with the following antibodies: anti-Flag (Abcam), anti-GFP (Sigma-Aldrich), anti-mDia2, anti-HDAC6, anti-GAPDH (Cell Signaling), and anti-Acetyl-lysine (Upstate Biotechnology).

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Figure S1. Inhibition of HDAC6 impairs enucleation. (A) Immunostaining of HDAC6 in cultured TER119- mouse fetal progenitors treated with TubA or infected with retrovirus harboring HDAC6 shRNA as indicated. The cells were fixed at 24 h and then immunostained with anti-HDAC6 conjugated with Alexa Fluor 488 and DAPI. Scale bar is 10µm. (B) TER119- mouse fetal progenitors were treated with DMSO (C) or TubA for 24 or 48 h and the cell lysate was subjected to western blot with antibodies as indicated. (C) The HDAC6 gene expression was determined by RT-real time PCR and normalized with the expression of GAPDH. Flow cytometric analysis of cultured TER119- mouse fetal progenitors at 48h treated with DMSO or sirtinol (50µM) (D) and nicotinamide (NAM) (5mM) (E).

Figure S2





(A) Quantification of F-actin fluorescence intensity in differentiating TER119mouse fetal progenitors treated with DMSO or TubA (ref. Fig. 2B). (B) Quantification polarized F-actin in cells treated with DMSO or TubA. The error bars represent mean +/-s.d. (n>=30), ****p<0.0001.

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Figure S3. Inhibition of HDAC6 impairs cell proliferation and cytokinesis. (A) Immunostaining of F-actin in cultured uninduced TER119- mouse fetal progenitors treated with DMSO or TubA for 18 h. Scale bar is 10µm. (B) DMSO or 10 um TubA was added to 1.2 X10⁶ cultured TER119- mouse fetal progenitors and cell number was counted 24 h after treatment. (C) $3X10^5$ NIH 3T3 cells were seeded and DMSO or TubA was added 24 h after seeding. The cell number was counted 48 h after seeding. The data presents as mean +/- s.d. (n=3), *p<0.05. (D) NIH 3T3 cells were treated with DMSO or 10 uM TubA for 24 h. Cells were fixed and immunostained with phalloidin-Texas red and DAPI. Scale bar is 5µm.

Figure S4

Q9NSV4	659	MTENCFWIKVNENKYEN VDLLCKLENTFCCQQKERREEEDIEEKKSI-KKKIKELKFLDSKIAQNLSIFLS-SF	730
Q9Z207	638	MSENCFWIKVNENKYEN RDLLCKLENTFCCQEKEKRNTNDFDEKKVI-KKRMKELKFLDPKIAQNLSIFLS-SF	709
060610	792	LSQDCFWTKVKEDRFEN NELFAKLTLTFSAQTKTSKAKKDQEGgeEKKSVQKKKVKELKVLDSKTAQNLSIFLG-SF	867
008808	775	LSQDCFWTKVKEDRFEN NELFAKLTLAFSAQTKTSKAKKDQEGGeEKKSVQKKKVKELKVLDSKTAQNLSIFLG-SF	850
NP 476981	624	MSDKAFWVKCQEDKLAQ DDFLAELAVKFSSKPVKKEQKDAVDKPTTLTKKNVDLRVLDSKTAQNLAIMLGqSL	696
CAA96179	1368	CTDNSIWGTGKAEKFAD[4]KGVLADLEKAFAAREIKSLASKRKEDLQKITFLSRDISQQFGINLH-MY	1436
09NSV4	731	-RVPYEEIRMMILEVDETRL-AESMIONLIK HLPDOEOLNSLSOFKS EYSNLCEPEO-FVVVMSNV-KRLRPR	799
~ 09Z207	710	-RVPYEKIRTMILEVDETOL-SESMIONLIK HLPDECOLKSLSOFRS DYNSLCEPEO-FAVVMSNV-KRLRPR	778
060610	868	-RMPYOEIKNVILEVNEAVL-TESMIONLIK OMPEPEOLKMLSELKD EYDDLAESEO-FGVVMGTV-PRLRPR	936
008808	851	-RMPYOEIKNVILEVNEAVL-TESMIONLIK OMPEPEOLKMLSELKE EYDDLAESEO-FGVVMGTV-PRLRPR	919
NP 476981	697	KHLSVEOTKICLLRCDTDIL-SSNILOOLO YLPPDEHLKRLOEIKA KGEPLPDIEO-FRATIGEI-KRLSPR	766
CAA96179	1437	SSLSVADLUKKTINCORDELATDSVETISKI 31VADVSTNWEGVENIED 61 DENDLORADOIYLOLMVNLSSVWGSR	1528
CARJOITS	1457	SODOANDARCHARDERGIISAABEDOK(IS)INI DIDMEGARCHERG(OIDIMEDAKKHERINARDEDIMOSK	1020
OBNEWA	900	I SATI EVI OFFOUNNI VDDIMAUSTACEF IVVSVSESVI I FIWI I MONYMUACSBNAOTECENI SSI OVI VDTVSADOV	070
008207	770	LATIEN OFFICE UNITED INVESTIGATION OF THE STATE OF THE ST	075
050510	007	LSATLERINGE BEVONNTREDIMAVSTACEETRKSRESSLIEDUNGN MINASSRAAVTEGEDISSLCALAD INSADOR	1010
000010	937	LINATIERELOGESEQVENTINEETVSVTAACEELERKSESESILLEETTILLVON IMMAGSRINAGAFGENTSELCELERTINSTOOD	1016
008808	920	LNAT LE KLQVENTKPETVS VTAACEELKKSENESSLEETTLVGN MNAGSKNAGAFGENTSELCKLKDTKSADQK	999
<u>NP_476981</u>	767	LHNLNFKLTYADMVQDIKPDIVAGTAACEEIRNSKKFSKILELILLLGNYMNSGSKNEAAFGFEISYLTKLSNTKDADNK	846
CAA96179	1529	MRALTVVTSYEREYNELLAKLRKVDKAVSALQESDNLRNVFNVILAVGNFMNDTSKQAQGFKLSTLQRLTFIKDTTNS	1606
Q9NSV4	880	TTLLHFLVEICEEKYPDILNFVDDLEPLDKASKVSVETLEKNLRQMGRQLQQLEKELETFPP PEDLHDKFVTKMSRF	956
<u>Q9Z207</u>	859	TTLLHFLVDVCEEKHADILHFVDDLAHLDKASRVSVEMLEKNVKQMGRQLQQLEKNLETFPP PEDLHDKFVIKMSSF	935
060610	1017	MTLLHFLAELCENDYPDVLKFPDELAHVEKASRVSAENLQKNLDQMKKQISDVERDVQNFPA ATDEKDKFVEKMTSF	1093
008808	1000	MTLLHFLAELCENDHPEVLKFPDELAHVEKASRVSAENLQKSLDQMKKQIADVERDVQNFPA ATDEKDKFVEKMTSF	1076
NP_476981	847	QTLLHYLADLVEKKFPDALNFYDDLSHVNKASRVNMDAIQKAMRQMNSAVKNLETDLQNNKV PQCDDDKFSEVMGKF	923
CAA96179	1607	MTFLNYVEKIVRLNYPSFNDFLSELEPVLDVVKVSIEQLVNDCKDFSQSIVNVERSVEIGNL[4]KFHPLDKVLIKTLPV	1687
Q9NSV4	957	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK-K/SVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA	1035
<u>Q9NSV4</u> <u>Q9Z207</u>	957 936	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK <mark>K</mark> /SVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDMK-K/SVEEFFNDLNNFRTSFMLALKENIKKREAAEKEKRARIAKERA	1035 1014
<u>Q9NSV4</u> <u>Q9Z207</u> 060610	957 936 1094	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK <mark>K</mark> YSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDMK KYSVEEFFNDLNNFRTSFMLALKENIKKREAAEKEKRARIAKERA VKDAQEQYNKLRMMHSNMETLYKELGEYFLFDPK KJSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA	1035 1014 1172
<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u>	957 936 1094 1077	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK <mark>K</mark> YSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDMK KYSVEEFFNDLNNFRTSFMLALKENIKKREAAEKEKRARIAKERA VKDAQEQYNKLRMMHSNMETLYKELGEYFLFDPK KJSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA VKDAQEQYNKLRMMHSNMETLYKELGDYFVFDPK KJSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA	1035 1014 1172 1155
<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> NP_476981	957 936 1094 1077 924	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK KYSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDMK KYSVEEFFNDLNNFRTSFMLALKENIKKREAAEKEKRARIAKERA VKDAQEQYNKLRMMHSNMETLYKELGEYFLFDPK KJSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA VKDAQEQYNKLRMMHSNMETLYKELGDYFVFDPK KJSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AEECRQQVDVLGKMQLQMEKLYKDLSEYYAFDPS KYTMEEFFADIKTFKDAFQAAHNDNVRVREELEKKRRLQEAREQS	1035 1014 1172 1155 1002
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<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u>	957 936 1094 1077 924 1688 1036 1015 1173 1156 1003 1768 1110	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK KVSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDK KVSVEEFFNDLNNFRTSFMLALKENIKKREAEEKEKRARIAKERA VKDAQEQYNKLRMHSNMETLYKELGEYFLFDPK KVSVEEFFNDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDPS KVTMEEFFADIKTFKDAFQAAHNDNVRVREELEKKRRLQEAREQS LPEARKKGDLLEDEVKLTIMEFESLMHTYGEDSGKKTAKISFFKKFADFINEYKKAQAQNLAAEEEERLYIKHKKIVEEQ ERERLERQQKKKRLLEMKTEGDET GVMDNLLEALQSGAAF RDRRKRTPMFKDVRQSLSPMSQRPVLKVCNHENQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF RDRRKRTPKLKDIRQSLSPMSQRPVLKVCNHENQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF R-RKRGPR	1035 1014 1172 1155 1002 1767 1109 1088 1223 1206 1080 1823 1185
<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9SV4</u> <u>Q9Z207</u>	957 936 1094 1077 924 1688 1036 1015 1173 1156 1003 1768 1110 1089	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK KVSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDK KVSVEEFFNDLNNFRTSFMLALKENIKKREAEEKEKRARIAKERA VKDAQEQYNKLRMHSNMETLYKELGEYFLFDPK KVSVEEFFNDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA VKDAQEQYNKLRMHSNMETLYKELGDYFVFDFK KVSVEEFFNDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQQVDVLGKMQLQMEKLYKDLSEYYAFDPS KVTMEEFFADIKTFKDAFQAAHNDNVRVREELEKKRRLQEAREQS LPEARKKGDLLEDEVKLTIMEFESLMHTYGEDSGCK TAKISFFKKFADFINEYKKAQAQNLAAEEEERLYIKHKKIVEEQ ERERLERQQKKKRLLEMKTEGDET GVMDNLLEALQSGAAF RDRRKRTPMFKDVRQSLSPMSQRPVLKVCNHENQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF RDRRKRTPKLKDIRQSLSPMSQRPVLKVCNHENQ EKERLEKQQKREQLIDMNAEGDET GVMDSLLEALQSGAAF RRKRGPR	1035 1014 1172 1155 1002 1767 1109 1088 1223 1206 1080 1823 1185 1163
<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u>	957 936 1094 1077 924 1688 1036 1015 1173 1156 1003 1768 1110 1089 1224	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK KVSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDK KVSVEEFFNDLNNFRTSFMLALKENIKKREAEEKEKRARIAKEAA VKDAQEQYNKLRMHSNMETLYKELGEYFLFDFK KVSVEEFFNDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEFFFMDLKTFKDAFQAAHNDNVRVREELEKKRRLQEAREQS LFEARKKGDLLEDEVKLTIMEFESLMHTYGEDSGK FAKISFFKKFADFINEYKKAQAQNLAAEEEERLYIKHKKIVEEQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF RDRRKRTPMFKDVRQSLSPMSQRPVLKVCNHENQ EKERLEKQQKREQLIDMNAEGDET GVMDSLLEALQSGAAF RRKRGPR	1035 1014 1172 1155 1002 1767 1109 1088 1223 1206 1080 1823 1185 1163 1269
<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u>	957 936 1094 1077 924 1688 1036 1015 1173 1156 1003 1768 1110 1089 1224 1207	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK KVSVEDFLTDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDK KVSVEEFFNDLNNFRTSFMLALKENIKKREAEEKEKRARIAKEAA VKDAQEQYNKLRMHSNMETLYKELGEYFLFDFK KVSVEEFFNDLNNFRTSFMLALKENIKKREAEEKEKRARIAKEKA VKDAQEQYNKLRMHSNMETLYKELGDYFVFDFK KVSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECCRQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AEECRQQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSVEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AEECRQQVKKRLLEMKTEGDET GVMDNLLEALQSGAAF RDRKKTPMPKDVRQSLSPMSQRPVLKVCNHENQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF RDRKKTPMKLKDIRQSLSPMSQRPVLKVCNHENQ EKERLEKQQKREQLIDMNAEGDET GVMDSLLEALQSGAAF RRKRGPR	1035 1014 1172 1155 1002 1767 1109 1088 1223 1206 1080 1823 1185 1163 1269 1252
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<u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u> <u>Q9NSV4</u> <u>Q9Z207</u> <u>060610</u> <u>008808</u> <u>NP_476981</u> <u>CAA96179</u>	957 936 1094 1077 924 1688 1036 1015 1173 1156 1003 1768 1110 1089 1224 1207 1081 1824	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDKK KVSVEDFFDDLNNFRTTFMQAIKENIKKREAEEKERRVRIAKELA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDK KVSVEEFFDDLNNFRTTSMLALKENIKKREAAEKERRARIAKERA VKDAQEQYNKLRMMHSNMETLYKELGEYFLFDFK KVSVEEFFDDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKERA AEECRQQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSEEFFDDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AEECRQQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSEEFFDDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AEECRQQVDVLGKMQLQMEKLYKDLSEYYAFDFS KVSEEFFDDLHVFKDAFQAAHNDNVRVREELEKKRRLQEAREQS LPEARKKGDLLEDEVKLTIMEFESIMHTYGEDSG KVAKISFFKKFADFINEYKKAQAQNLAAEEEERLYIKHKKIVEEQ ERERLERQQKKKRLLEMKTEGDET GVMDNLLEALQSGAAF RDRKRTPMFKDVRQSLSPMSQRPVLKVCNHENQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF RDRRKTPKLKDIRQSLSPMSQRPVLKVCNHENQ EKERLEKQQKREQLIDMNAEGDET GVMDSLLEALQSGAAF RRKRGPR	1035 1014 1172 1155 1002 1767 1008 1223 1206 1080 1823 1185 1163 1269 1252 1091 1901
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Q9NSV4 Q9Z207 060610 008808 NP_476981 CAA96179 Q9NSV4 Q9Z207 060610 008808 NP_476981 CAA96179 Q9NSV4 Q9Z207 060610 008808 NP_476981 CAA96179 Q9NSV4 Q9Z207 060610 008808 NP_476981	957 936 1094 1077 924 1688 1015 1173 1156 1003 1768 1110 1089 1224 1207 1081 1824 1186 1164 1270 1253	VISAKEQYETLSKLHENMEKLYQSIIGYYAIDVK KISVEDFITDLNNFRTTFMQAIKENIKKREAEEKEKRVRIAKEA VISANEQYEKLSTLLGSMTQLYQSIMGYYAVDMK KISVEEFFNDLNNFRTSFMLALKENIKKREAAEKEKRARIAKEA VKDAQEQYNKLRMMHSNMETLYKELGEYFLFDEK KLSVEEFFNDLNNFRTSFMLALKENIKKREAAEKEKRARIAKEA AECRQQVDVLGKMQLQMEKLYKDLSEYYAFDPS KISVEEFFNDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKA AECRQQVDVLGKMQLQMEKLYKDLSEYYAFDPS KITMEEFFADIKTFKDAFQAAHNDNVRVREELEKKRRLQEAREQS LEEARKKGDLLEDEVKLITIMEFESLMHTYGEDGGKAFAKISFFKKFADFINEVKKAQAQNLAAEEEERLYIKHKKVEEQ EKERLERQQKKKRLLEMKTEGDET GVMDNLLEALQSGAAF RDRKKTPMPKDVRQSLSPMSQRPVLKVCNHENQ EKERLERQQEKKRLLEMKTEGDET GVMDSLLEALQSGAAF RKKRGPRQANR EKERLEKQQKREQLIDMNAEGDET GVMDSLLEALQSGAAF RKKRGPR	1035 1014 1172 1155 1002 1767 1088 1223 1206 1080 1823 1185 1163 1269 1252 1091 1901

Figure S4. K970 in FH2 domain of mouse mDia2 is highly conserved. FH2 domain of formin protein alignment among species. Conserved k970 is marked by the black box. Red shows highly conserved residues and blue shows less conserved residues. Unaligned residues are shown in grey. Sequence showed are Homo sapiens mDia2 (Q9NSV4), Mus musculus mDia2 (Q9Z207), Homo sapiens mDia1 (O60610), Mus musculus mDia1 (O08808), Drosophila melanogaster Diaphanous (NP_476981.1), and Saccharomyces cerevisiae Bni1 (CAA96179.1).



Figure S5. **Expression of mDia2 and mutants in knock down cells.** (A). TER119mouse fetal progenitors were infected with retrovirus harboring mDia2 shRNA with a GFP marker. The cells were fixed at 24 h and immunostained with anti-mDia2 conjugated with Alex-594 and DAPI. GFP indicates knockdown of endogenous mDia2 and mDia2 staining indicates endogenous mDia2. (B) The mDia2 gene expression in mDia2 knock down mouse fetal progenitors was determined by RT-real time PCR and normalized with the expression of GAPDH. (C) The mDia2 knock down cells were also co-infected with retrovirus that carrying shRNA resistant Flag tagged mDia2 WT, K970R or K970Q. The cells were fixed at 24 h and immunostained with anti-Flag conjugated with Alex-594 and DAPI. GFP indicates knockdown of endogenous mDia2 and Flag staining indicates re-expression of mDia2 mutants. Scale bar is 10µm.

Figure S6



Figure S6. **Expression of mDia2 and mutants in knock down cells.** TER119⁻ mouse fetal progenitors were co-infected with retrovirus harboring HDAC6 shRNA and retrovirus that carrying Flag tagged mDia2 K970R. The cells were fixed at 24 h and immunostained with anti-Flag conjugated with Texas red, anti-HDAC6 conjugated with Alexa Fluor 488, and DAPI. Scale bar is 10µm.