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online data

SUPPLEMENTARY ONLINE DATA

A novel non-canonical mechanism of regulation of MST3 (mammalian Sterile20-related kinase 3)

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NP_001027467.2      MAHSPVQSGLPGMQ-------------------------NLKADPEELFTKLEKIGKGS
NP_003567.2         -------------MDSRAQLWGLALNKRRATLPHPGGSTNLKADPEELFTKLEKIGKGS
Rat MST3            MAHSPVQSGLPGMQ-------------------------TLKADPEELFTKLEKIGKGS
NP_663440.1         MAHSPVQSGLPGMQ-------------------------NLKADPEELFTKLEKIGKGS
                                                           .******************

NP_001027467.2      FGEVFKGIDNRTQKVVAIKIIDLEEAEDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTK
NP_003567.2         FGEVFKGIDNRTQKVVAIKIIDLEEAEDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTK
Rat MST3            FGEVFKGIDNRTQKVVAIKIIDLEEAEDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTK
NP_663440.1         FGEVFKGIDNRTQKVVAIKIIDLEEAEDEIEDIQQEITVLSQCDSPYVTKYYGSYLKDTK
                    ************************************************************

NP_001027467.2      LWIIMEYLGGGSALDLLEPGPLDETQIATILREILKGLDYLHSEKKIHRDIKAANVLLSE
NP_003567.2         LWIIMEYLGGGSALDLLEPGPLDETQIATILREILKGLDYLHSEKKIHRDIKAANVLLSE
Rat MST3            LWIIMEYLGGGSALDLLEPGPLDEIQIATILREILKGLDYLHSEKKIHRDIKAANVLLSE
NP_663440.1         LWIIMEYLGGGSALDLLEPGPLDEIQIATILREILKGLDYLHSEKKIHRDIKAANVLLSE
                    ************************ ***********************************

NP_001027467.2      HGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGITAIELAR
NP_003567.2         HGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGITAIELAR
Rat MST3            HGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGITAIELAK
NP_663440.1         HGEVKLADFGVAGQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDSKADIWSLGITAIELAK
                    ************************************************************:

NP_001027467.2      GEPPHSELHPMKVLFLIPKNNPPTLEGNYSKPLKEFVEACLNKEPSFRPTAKELLKHKFI
NP_003567.2         GEPPHSELHPMKVLFLIPKNNPPTLEGNYSKPLKEFVEACLNKEPSFRPTAKELLKHKFI
Rat MST3            GEPPHSELHPMKVLFLIPKNNPPTLEGSYSRPLKEFVEACLNKEPSFRPTAKELLKHKFI
NP_663440.1         GEPPHSELHPMKVLFLIPKNNPPTLEGNYSKPLKEFVEACLNKEPSFRPTAKELLKHKFI
                    ***************************.**:*****************************

NP_001027467.2      LRNAKKTSYLTELIDRYKRWKAEQSHDDSSSEDSDAETDGQASGGSDSGDWIFTIREKDP
NP_003567.2         LRNAKKTSYLTELIDRYKRWKAEQSHDDSSSEDSDAETDGQASGGSDSGDWIFTIREKDP
Rat MST3            IRNAKKTSYLTELIDRYKRWKAEQSHEDSSSEDSDVETDSQASGGSDSGDWIFTIREKDP
NP_663440.1         IRNAKKTSYLTELIDRYKRWKAEQSHEDSSSEDSDVETDGQASGGSDSGDWIFTIREKDP
                    :************************:********.***.********************

NP_001027467.2      KNLENGALQPSDLDRNKMKDIPKRPFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRG
NP_003567.2         KNLENGALQPSDLDRNKMKDIPKRPFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRG
Rat MST3            KNLENGTLQPSDLERNKMKDFPKRPFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRG
NP_663440.1         KNLENGTLQLSDLERNKMKDIPKRPFSQCLSTIISPLFAELKEKSQACGGNLGSIEELRG
                    ******:** ***:******:****************************************

NP_001027467.2      AIYLAEEACPGISDTMVAQLVQRLQRYSLSGGGTSSH 431
NP_003567.2         AIYLAEEACPGISDTMVAQLVQRLQRYSLSGGGTSSH 443
Rat MST3            AIYLAEEACPGISDTMVAQLVQRLQRYSLSGGGASAH 431
NP_663440.1         AIYLAEEACPGISDTMVAQLVQRLQRYSLSGGGASAH 431
                    *********************************:*:*
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Figure S1 MST3 alignments in *H. sapiens*, mouse and rat

GenBank® accession numbers are NP_001027467.2 for *H. sapiens* 431 residue MST3 [note that this is shown as shown as MST3b on the NCBI database (http://www.ncbi.nlm.nih.gov/protein/)], NP_003567.2 for *H. sapiens* 443 residue MST3b (shown as MST3a on the NCBI database) and NP_663440.1 (431 residues) for mouse. The rat sequence was derived from our cDNA sequence, EU371958.1, but now also see NP_001120966.1 (431 residue MST3), deposited on 14-09-2011. ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used to align the sequences. As implied, the attributions in the NCBI database are confused. The earliest reference associated with the 443 residue NP_003567.2 entry [1] in fact refers to the 431 residue protein, whereas the earliest references associated with the NP_001120966.1 entry refer to both the 431 residue [1] and the 443 residue [2] proteins.

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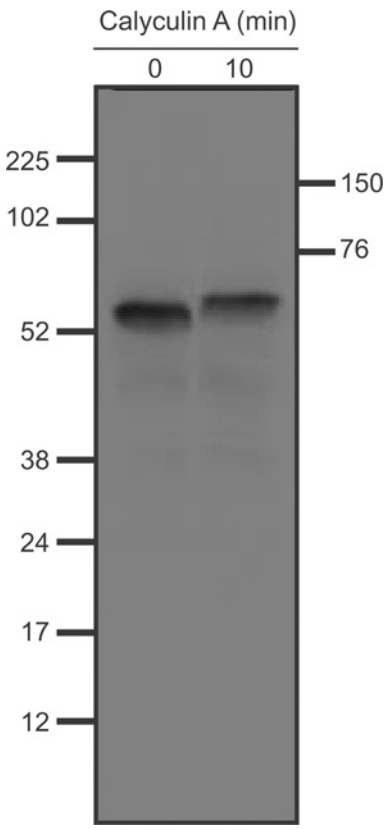

Figure S2 Exposure of myocytes to calyculin A results in the appearance of only a single 58 kDa FLAG–MST3 band

Myocytes were infected with AdV–FLAG–MST3(FL). They were either not stimulated or were exposed to calyculin A (100 nM for 10 min), and extracts were prepared. Proteins were separated by SDS/PAGE followed by immunoblot analysis with anti-FLAG antibodies. The numbers on the sides of the immunoblot signify the positions of the molecular-mass markers in kDa.

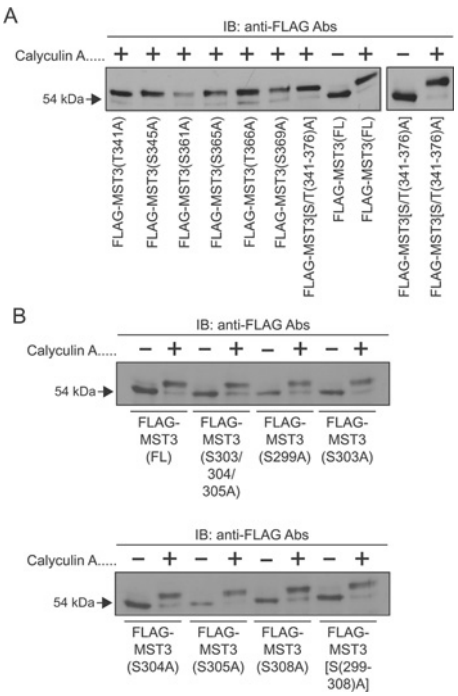

Figure S3 Alanine scanning of the FLAG–MST3(341–376) and FLAG–MST3(299–308) regions

Myocytes were infected with the appropriate AdV vectors. They were either unstimulated or were exposed to calyculin A (100 nM for 10 min), and extracts were prepared. Proteins were separated by SDS/PAGE followed by immunoblot (IB) analysis with anti-FLAG antibodies. Immunoblots are representative of experiments repeated at least twice with different myocyte preparations. Calyculin A-induced reductions in FLAG–MST3(FL) are shown for comparison. (**A**) Individual (left-hand panel) mutation of each of the six serine/threonine residues in the FLAG–MST3(341–376) region or combined mutation of all six serine/threonine residues (right-hand panel) did not affect the ability of calyculin A to reduce the mobility of FLAG–MST3. (**B**) Individual mutation of each of the five serine residues in the FLAG–MST3(299–308) region or combined mutation of FLAG–MST3(S303/304/305) and FLAG–MST3(S299/303/304/305/308) did not affect the ability of calyculin A to reduce the mobility of FLAG–MST3.

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