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SUPPLEMENTARY MATERIAL

corresponding to:

Hippo signaling components, Mst1 and Mst2, act as a switch between self-renewal and differentiation in *Xenopus* hematopoietic and endothelial progenitors

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Α

hMST1 mMst1 xtMst1 hMST2 mMst2 xtMst2	1 1 1 1 1	METVQLRNPPRRQLKKLDEDSLTKQPEEVFDVLEKLGEGSYGSVYKAIHKETGQIVAIKQVPVESDLQEIIKEISIMQQCDSPHVVKYYG METVQLRNPPRRQLKKLDEDSLTKQPEEVFDVLEKLGEGSYGSVYKAIHKETGQIVAIKQVPVESDLQEIIKEISIMQQCDSHVVKYYG MEQPPAPKSKLKKLSEDSLTKQPEEVFDVLEKLGEGSYGSVYKAIHKESGQVVAIKQVPVESDLQEIIKEISIMQQCDSPYVVKYYG MEQPPAPKSKLKKLSEDSLTKQPEEVFDVLEKLGEGSYGSVFKAIHKESGQVVAIKQVPVESDLQEIIKEISIMQQCDSPYVVKYYG MEQPPAPKSKLKKLSEDSLTKQPEEVFDVLEKLGEGSYGSVFKAIHKESGQVVAIKQVPVESDLQEIIKEISIMQQCDSPYVVKYYG MET N PLKKLSEDSLTKQPEEVFDVLEKLGEGSYGSVFKAIHKESQVVAIKQVPVESDLQEIIKEISIMQQCDSPYVKYYG MET N PLKKLSEDSLTKQPEEVFDVLEKLGEGSYGSVFKAIHKESQVVAIKQVPVESDLQEIIKEISIMQQCDSPVVKYYG	90 90 90 87 87 86
nMST1 mMst1	91 91	SYFKNTDLW IVMEYCGAGSVSD I IRLENKTLTEDE IAT ILQSTLKGLEYLHFMRK IHKD IKAGN ILLNTEGHAKLADFGVAGQLTDTMAK SYFKNTDLW IVMEYCGAGSVSD I TRLENKTLTEDE IAT ILQSTLKGLEYLHFMRK IHKD IKAGN ILLNTEGHAKLADFGVAGQLTDTMAK	180 180
xtMst1	91	SYFKN TDLW IVMEFCGGGS I SD I IRLRKOTLKEDETAT IL OSTLKGLEYLHFMRK IHRD IKAGN ILLN SEGTAKLADFGVAGOLTDTMAK	180
hMST2	88	SYFKNTDLW IVMEYCGAG SV SD I IRLRNKTL IEDE IAT ILK STLKGLEYLHFMRK IHRD IKAGN ILLNTEGHAKLADFGVAGQLTDTMAK	177
mMst2	88	SYFKNTDLW IVMEYCGAG SV SD I IRLRNKTLTEDE IAT II <mark>K</mark> STLKGLEYLHFMRK IHRD IKAGN ILLNTEGHAKLADFGVAGQLTDTMAK	177
xtMst2	87	<u>SYFKNTDLWIVMEYCGAGSVSDIIRLRNKTLTEEEIATTLRSTLKGLEVLHFMRKIHRDIKAGNILLNTEGHAKLADFGVAGQLTDTMAK</u> SYFKNTDLWIVMEYCGAGSVSDIIRLRNKTLTEDEIATIL STLKGLEYLHFMRKIHRDIKAGNILLNTEGHAKLADFGVAGQLTDTMAK	176
hMST1	181	RN TV IG TPFWMA PEVIQE IG YN CVAD IW SLG IT <mark>A</mark> IEMAEG KPPYAD IHPMRA IFM IPTN PPPTFR KPELW SD <mark>N FTDFV KOCLVK S</mark> PEORA	270
mMst1	181	RNTVIGTPFWMAPEVIQEIGYNCVADIWSLGITAIEMAEGKPPYADIHPMRAIFMIPTNPPPTFRKPELWS <u>DNFMDFVK</u> QCLVKSPE <u>Q</u> RA	270
xtMst1	181	RNTV IGTPFWMAPEV IQE IGYNCVAD IWSLG ITA IEMAEGKPPYAE IHPMRA IFM IPSNPPPTFRKPELWSKDFVDF INLCLVKNPBLRS	270
nMS12 mMct2	178	RNTVIGTPFWMAPEVIQEIGYNCVADIWSLGITSIEMAEGKPPYADIHPMRAIFMIPTNPPPTFRKPELWSDDFTDFVKKCLVKNPEQKA	267
xtMst2	177	KNTV 1G TERMMAPEV 1QE 1G IN VAD IWSIG IT SIEMAEGKPPIAD I HPMRA IPM IPT NPPPTR KNPLIW SDUFTDFV KKCLV KSPEQKA V RNTV TGTPEWMAPEV 1QE FGVNCVAD IWSIG TT SIEMAEGKPEVAL THONEA IFM TENDDDTERKER I. WEIFFNFEVKCLV KNDE (DE I	267
		RNTVIGTPFWMAPEVIQEIGYNCVADIWSLGIT IEMAEGKPPYADIHPMRAIFMIPTNPPPTFRKPELWSD.FTDFVK CLVK PEQRA	
hMST1	271	TATQLLQHPFV <mark>R</mark> SAKGVS ILRDL INEAMDVKLKRQESQQREVDQDDEEN SEE <mark>DEMDS</mark> GTMVRAVGDEMGTVRVASTMTDGANTM IEHDDT	360
mMst1	271	TATQLLQHPFV <mark>K</mark> SAKGVSILRDLINEAMDVKLKRQEAQQREVDQDDEENSEEDEMDSGTMVRAAGDEMGTVRVASTMSGGANTMIEHGDT	360
xtMst1	271	SATELLQHPF IKTAKGESILRHL INEAQDAKLKRTELKQREVEPEEEENADEDEADVGTMVQAGSKDLNTMKEFSTMNEAADCTMVEKOK	360
hMS12 mMct2	268	TATOLLOHPFIKNAKPVSILRDLITEAMEIKAKRHEEQQRELE - EEEENSDEDELDSHTMVKTSVESVGTMRATSTMSEGAQTMIEHNST	356
xtMst2	267	TATUCIONSE TANAKEVSTIDUI TABAME IKAKABEVQABLE - BEBENSUBUBUBUBUKTISSE SVGTMAATSINSBAQIM LENST	355
AUNTE		TATQLLQHPFIK AK VSILRDLI EAMD.K.KR E QQRE.EQEEEENS.EDE.DS TMVGTMR. STMSEGA.TMIEH T	
hMST1	361	-LPSQLGTMVINAEDEEEEGTMKRRDETMQPAKPSFLEYFEQKE-KENQINSFGKSVPGPLKNSSDWKIPQDGDYEFL	436
mMst1	361	-LPSQLGTMVINTEDEEEEGTMKRRDETMQPAKPSFLEYFEQKE-KENQINSFGKNVSGSLKNSSDWKIPQDGDYEFL	436
xtMst1	361	-LNTQMGTMLINDEDEEETGTMKQCTEPVQPAKPSFLEYFEQKENQFGTPEKTTPAPSTDPSEWKIPLNGDYSFL	434
hMST2	357	MLESDLGTMV IN SEDEEEEDGTMKRNATSPQVQRPSFMDYFDKQDFKNKSHENCNQNMHEPFPMSKNVFPDNWKVPQDGDFDFL	440
rtMst2	356	MLESDLGTMVINSEEBEBEBEBEBEDGTMKKNATSPQVQKPSTMDITDKQDTKNASHENCDQSMKEPGPMSNSVFPDNWKVPQDGPDFL MLFSDLGTMVINSEDEFFF——FRGTMKENATSQVQKPSTMDITDKQDTKNASKSDENCNONIHFOVHTSKUVPDNWKVPQDGPDFL	440
AUNDEL		ML S LGTMVIN.EDEEEEEEEEE.GTMKR . Q .PSF .YFFK P S NVFPD WK.PQDGDFL	
hMST1	437	KSWTVEDLQKRLLALDPMMEQEIEEIRQKYQSKRQPILDAIEAKKRRQQNF 487	
mMst1	437	KSWTVEDLQKRLLALDPMMEQEMEEIRQKYRSKRQPILDAIEAKKRRQQNF 487	1
xtMst1	435	kdwsvtelqlrlnsldpmmeqeieeinhkvqakropileaieskkrrqqnf 485	
nMST2	441		
xtMst2	443		
an en regelieter		K S.EELQ RL ALDPMME EIEE.RQ.Y AKROPILDA .AKKRRQQNF	
B			

Method: Neighbor Joining; Best Tree; tie breaking = Systematic Distance: Uncorrected ("p") Gaps distributed proportionally



Supplemental Fig. S1. Multiple protein sequence alignment of Mst. (A) The dark or light background highlights identical or similar residues, respectively. The domains of Mst2 are boxed using the following colors: red, kinase domain; blue, caspase cleavage site; light green, SARAH domain. (B) The phylogenetic tree of Mst proteins was clearly classified using the indicated Xenopus tropicalis Mst genes as homologs of mammalian Mst1 and Mst2, respectively. Identity scores (%) or similarity scores (%) are shown in parentheses at the right side of the panel. The following sequences were used: Hippo (NP_611427); hMST1 (NP_006273); hMST2 (NP_006272); mMst1 (NP_067395); mMst2 (NP_062609); xtMst1 (NP_989249); xtMst2 (NP_001090665); zMst2 (NP_955966); hMST, human MST; mMst, mouse Mst; xtMst, Xenopus tropicalis Mst; zMst, zebrafish Mst.



Supplemental Fig. S2. Loss of function phenotype was rescued by *Mst1 or Mst2 DNA* injection. (A-E) *After injection of 5 ng of standard control MO (Std. MO) or Mst1/2 MO (Mst1t+2s MO) into the animal pole at the 2-cell stage, embryos were injected with 20 pg of LacZ (pENL), Mst1 (pE-Mst1), or Mst2 (pE-Mst2) DNA into the vegetal pole. Coinjection of Mst1/2 MO and Mst1, or Mst2 DNA partially rescued morphological abnormality as compared to phenotype of Mst1/2 morphant (see also Fig.2). Injected embryos were cultured until stage 38. Injected MO is indicated in the upper right of each panel. Injected DNA is indicated in the lower right. (F) RT-qPCR analysis of β-globin in embryos coinjected with MO and DNA. The coinjection of standard control MO (Std. MO) and LacZ DNA did not alter the expression level of β-globin. In Mst1/2 MO and LacZ DNA-injected embryos, expression of β-globin was decreased (see also Fig. 3). As compared to LacZ DNA, Mst1, or Mst2 DNA slightly rescued decreased β-globin expression of Mst1/2 morphant. Expression levels were normalized relative to ODC. Values represent means + SEM of three independent experiments. (G) The embryonic vascular network was formed in standard control MO and LacZ DNA-coinjected embryo. (H) Impaired vascular formation in the Mst1/2 knockdown embryo could not be rescued by injection of LacZ DNA. (D,E) Coinjection of Mst1/2 MO and Mst1, or Mst2 DNA partially rescued abnormal vascular formation as compared to phenotype of Mst1/2 MO and LacZ DNA-coinjected embryos. Sample numbers are indicated in the upper right of each panel. Developmental stage is indicated in the lower left. Injected MO and DNA are indicated in the lower right all panels.*