THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

doi: 10.1387/ijdb.130010st

SUPPLEMENTARY MATERIAL

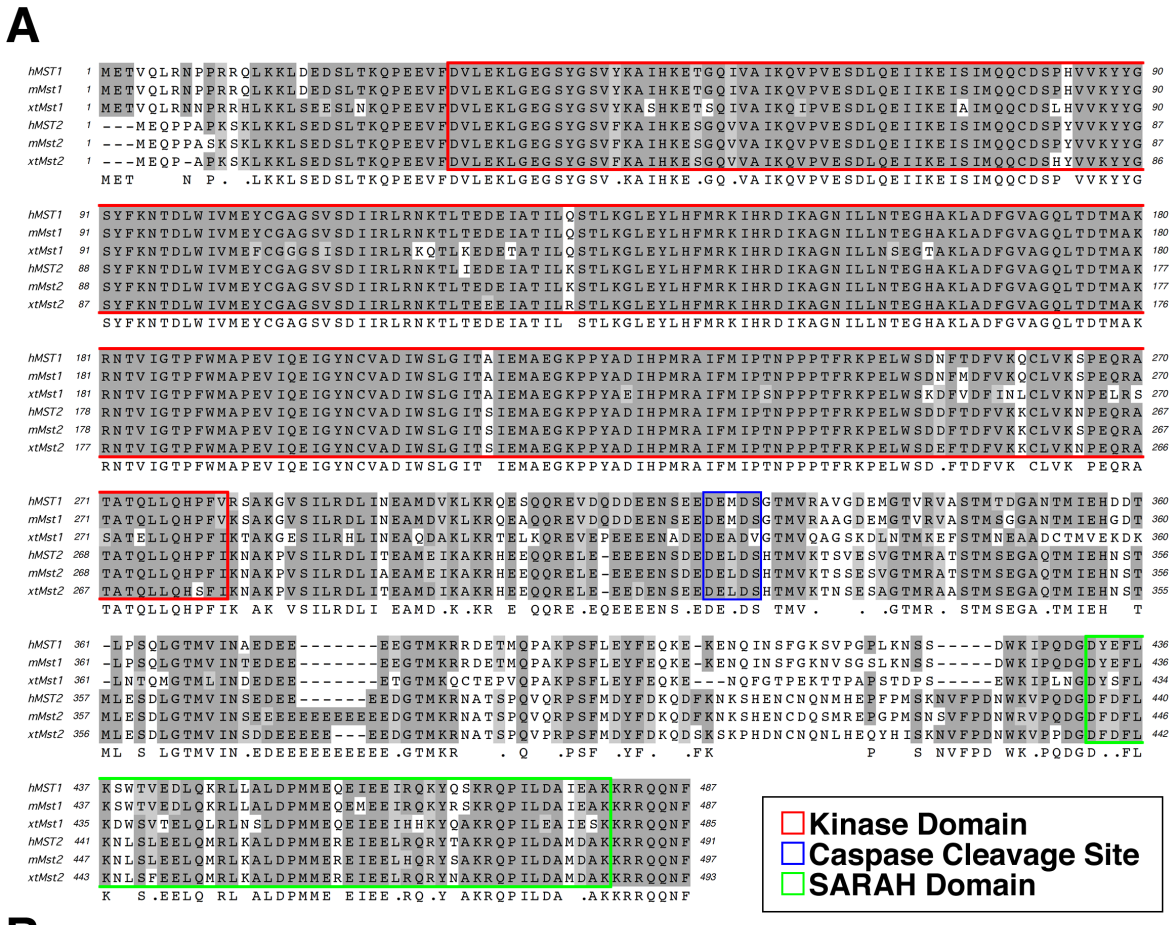
corresponding to:

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

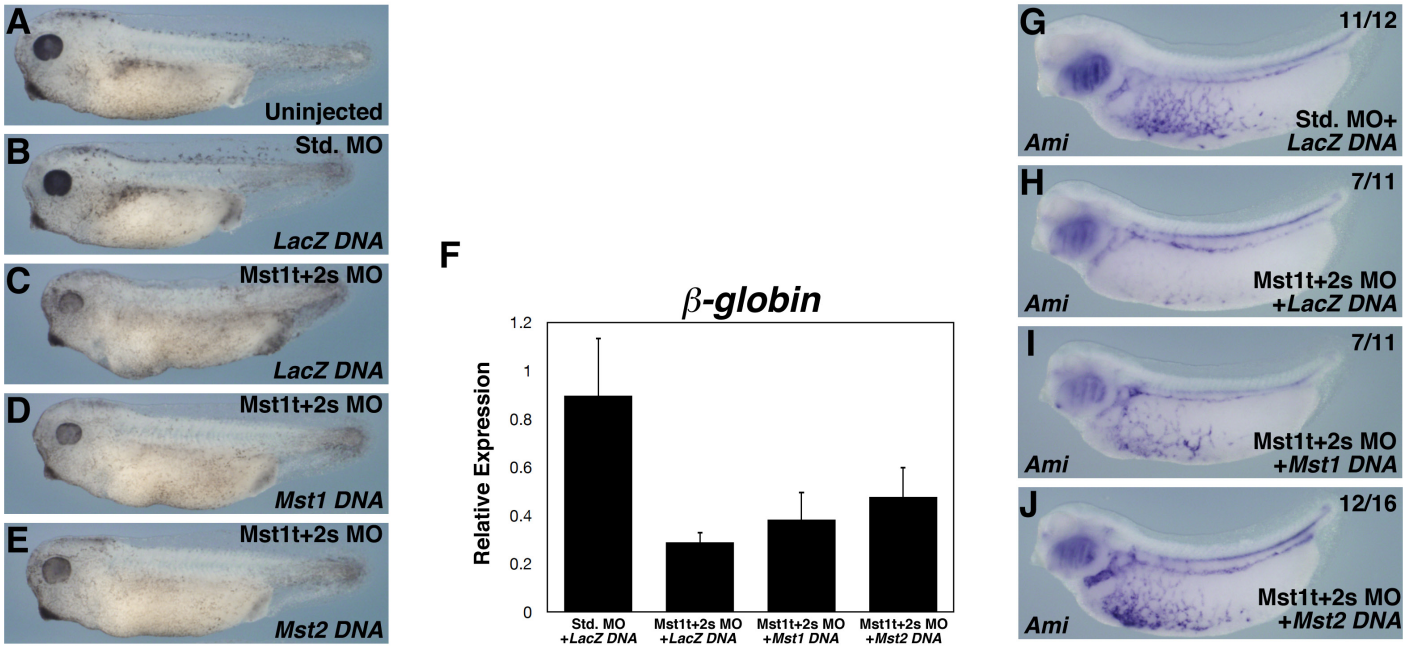
SUSUMU NEJIGANE, SHUJI TAKAHASHI, YOSHIKAZU HARAMOTO, TATSUO MICHIUE and MAKOTO ASASHIMA

***Address correspondence to:** Shuji Takahashi. Komaba Organization for Educational Excellence, College of Arts and Sciences, University of Tokyo, 3-8-1 Komaba, Meguroku, Tokyo 153-8902, Japan. Fax: +81-3-5465-8906. Tel: +81-3-5465-8908. E-mail: shujit@csls.c.u-tokyo.ac.jp

Full text for this paper is available at: http://dx.doi.org/10.1387/ijdb.130010st



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.*