

**SUPPLEMENTARY MATERIAL**

**corresponding to:**

**The *mob as tumor suppressor (mats1)* gene is required for growth control in developing zebrafish embryos**

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**A**

<i>Dm_MATS</i>	1	M	D	F	L	F	G	S	R	S	S	K	T	F	K	P	K	N	I	P	E	G	T	H	Q	Y	D	L	M	K	H	A	A	A	T	L	G	S	G	N	L	R	N	A	44
<i>Dr_MOBKLI1A</i>	1	M	S	F	L	F	G	S	R	S	S	K	T	F	K	P	K	N	I	P	E	G	S	H	Q	Y	E	L	L	K	H	A	E	A	T	L	G	S	G	N	L	R	M	A	44
<i>Hs_MOBKLI1A</i>	1	M	S	F	L	F	G	S	R	S	S	K	T	F	K	P	K	N	I	P	E	G	S	H	Q	Y	E	L	L	K	H	A	E	A	T	L	G	S	G	N	L	R	M	A	44
<i>Dr_MOB4B</i>	1	M	S	F	L	F	G	N	R	S	S	K	T	F	K	P	K	N	I	P	E	G	S	H	Q	Y	E	L	L	K	H	A	E	A	T	L	G	S	G	N	L	R	M	A	44
<i>Hs_MOBKLI1B</i>	1	M	S	F	L	F	S	R	S	S	K	T	F	K	P	K	N	I	P	E	G	S	H	Q	Y	E	L	L	K	H	A	E	A	T	L	G	S	G	N	L	R	Q	A	44	
<i>Dr_MOBKLI1B-LIKE</i>	1	M	S	F	L	F	G	N	R	S	S	K	T	F	K	P	K	N	I	P	E	G	S	H	Q	Y	E	L	L	K	H	A	E	A	T	L	G	S	G	N	L	R	A	44	
<i>Dr_MOBKLI1B</i>	1	M	S	F	L	F	G	N	R	S	S	K	T	F	K	P	K	N	I	P	E	G	S	H	Q	Y	E	L	L	K	H	A	E	A	T	L	G	S	G	N	L	R	A	44	

<i>Dm_MATS</i>	45	V	A	L	P	D	G	E	D	L	N	E	W	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	E	F	C	T	E	E	T	C	G	I	M	S	88	
<i>Dr_MOBKLI1A</i>	45	V	M	L	P	D	G	E	D	L	N	E	W	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	D	F	C	T	E	E	S	C	P	L	M	S	88	
<i>Hs_MOBKLI1A</i>	45	V	M	L	P	E	G	E	D	L	N	E	W	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	D	F	C	T	E	E	S	C	P	V	M	S	88	
<i>Dr_MOB4B</i>	45	V	M	L	P	E	G	E	D	L	N	E	W	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	D	F	C	S	E	D	S	C	P	V	M	S	88	
<i>Hs_MOBKLI1B</i>	45	V	M	L	P	E	G	E	D	L	N	E	W	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	E	F	C	T	E	A	S	C	P	V	M	S	88	
<i>Dr_MOBKLI1B-LIKE</i>	45	V	M	L	P	E	G	E	D	L	N	E	W	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	E	F	C	T	E	V	K	C	S	V	M	S	88	
<i>Dr_MOBKLI1B</i>	45	V	M	L	P	E	G	E	D	L	N	G	W	I	A	V	N	T	V	D	F	F	N	Q	I	N	M	L	Y	G	T	I	T	E	F	C	T	E	V	K	C	S	V	M	S	88

<i>Dm_MATS</i>	89	A	G	P	K	Y	E	Y	H	W	A	D	G	L	T	V	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132
<i>Dr_MOBKLI1A</i>	89	A	G	P	K	Y	E	Y	H	W	A	D	G	T	N	I	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132
<i>Hs_MOBKLI1A</i>	89	A	G	P	K	Y	E	Y	H	W	A	D	G	T	N	I	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132
<i>Dr_MOB4B</i>	89	A	G	P	K	Y	E	Y	H	W	A	D	G	T	N	I	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132
<i>Hs_MOBKLI1B</i>	89	A	G	P	R	Y	E	Y	H	W	A	D	G	T	N	I	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132
<i>Dr_MOBKLI1B-LIKE</i>	89	A	G	P	R	Y	E	Y	H	W	A	D	G	T	N	I	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132
<i>Dr_MOBKLI1B</i>	89	A	G	P	R	Y	E	Y	H	W	A	D	G	T	N	I	K	K	P	I	K	C	S	A	P	K	Y	I	D	Y	L	M	T	W	W	Q	D	L	D	D	E	T	L	F	132

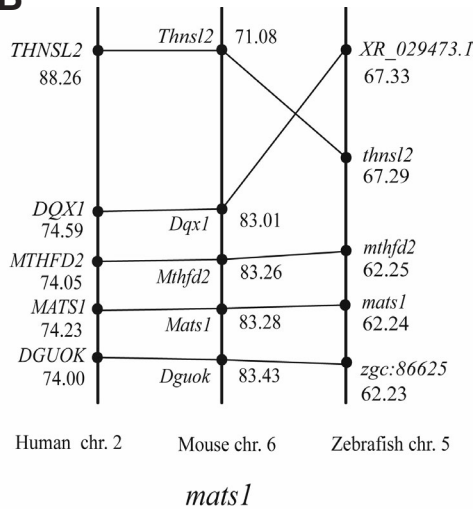
  

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<i>Dr_MOBKLI1A</i>	133	P	S	K	I	G	V	P	F	P	K	N	F	M	S	V	A	K	T	I	L	K	R	L	F	R	V	Y	A	H	I	Y	H	Q	H	F	S	V	I	Q	L	Q	E	176	
<i>Hs_MOBKLI1A</i>	133	P	S	K	I	G	V	P	F	P	K	N	F	M	S	V	A	K	T	I	L	K	R	L	F	R	V	Y	A	H	I	Y	H	Q	H	F	D	P	V	I	Q	L	Q	E	176
<i>Dr_MOB4B</i>	133	P	S	K	I	G	V	P	F	P	K	N	F	M	S	V	A	K	T	I	L	K	R	L	F	R	V	Y	A	H	I	Y	H	Q	H	F	D	A	V	M	Q	L	Q	E	176
<i>Hs_MOBKLI1B</i>	133	P	S	K	I	G	V	P	F	P	K	N	F	M	S	V	A	K	T	I	L	K	R	L	F	R	V	Y	A	H	I	Y	H	Q	H	F	D	S	V	M	Q	L	Q	E	176
<i>Dr_MOBKLI1B-LIKE</i>	133	P	S	K	I	G	V	P	F	P	K	N	F	M	S	V	A	K	T	I	L	K	R	L	F	R	V	Y	A	H	I	Y	H	Q	H	F	D	A	V	I	Q	L	Q	E	176
<i>Dr_MOBKLI1B</i>	133	P	S	K	I	G	V	P	F	P	K	N	F	M	S	V	A	K	T	I	L	K	R	L	F	R	V	Y	A	H	I	Y	H	Q	H	F	D	A	V	I	Q	L	Q	E	176

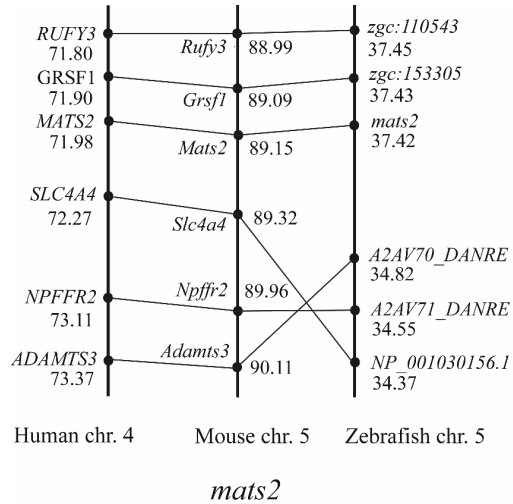
  

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<i>Dr_MOBKLI1A</i>	177	A	H	L	N	T	S	F	K	H	F	I	F	V	Q	E	F	N	L	I	D	R	K	E	L	A	P	L	Q	E	L	I	E	K	L	T	S	K	D	R	-	-	216	
<i>Hs_MOBKLI1A</i>	177	A	H	L	N	T	S	F	K	H	F	I	F	V	Q	E	F	N	L	I	D	R	R	E	L	A	P	L	Q	E	L	I	E	K	L	T	S	K	D	R	-	-	216	
<i>Dr_MOB4B</i>	177	A	H	L	N	T	S	F	K	H	F	I	F	V	Q	E	F	N	L	I	D	R	K	E	L	A	P	L	Q	E	L	I	E	R	L	T	T	K	D	R	-	-	216	
<i>Hs_MOBKLI1B</i>	177	A	H	L	N	T	S	F	K	H	F	I	F	V	Q	E	F	N	L	I	D	R	R	E	L	A	P	L	Q	E	L	I	E	K	L	G	S	K	D	R	-	-	216	
<i>Dr_MOBKLI1B-LIKE</i>	177	A	H	L	N	T	S	F	K	H	F	I	F	V	Q	E	F	N	L	I	D	R	R	E	L	A	P	L	Q	D	L	I	E	K	L	G	S	K	D	R	-	-	216	
<i>Dr_MOBKLI1B</i>	177	A	H	L	N	T	S	F	K	H	F	I	F	V	Q	E	F	N	L	I	D	R	R	E	L	A	P	L	Q	D	L	I	E	K	L	G	S	K	D	R	-	-	216	

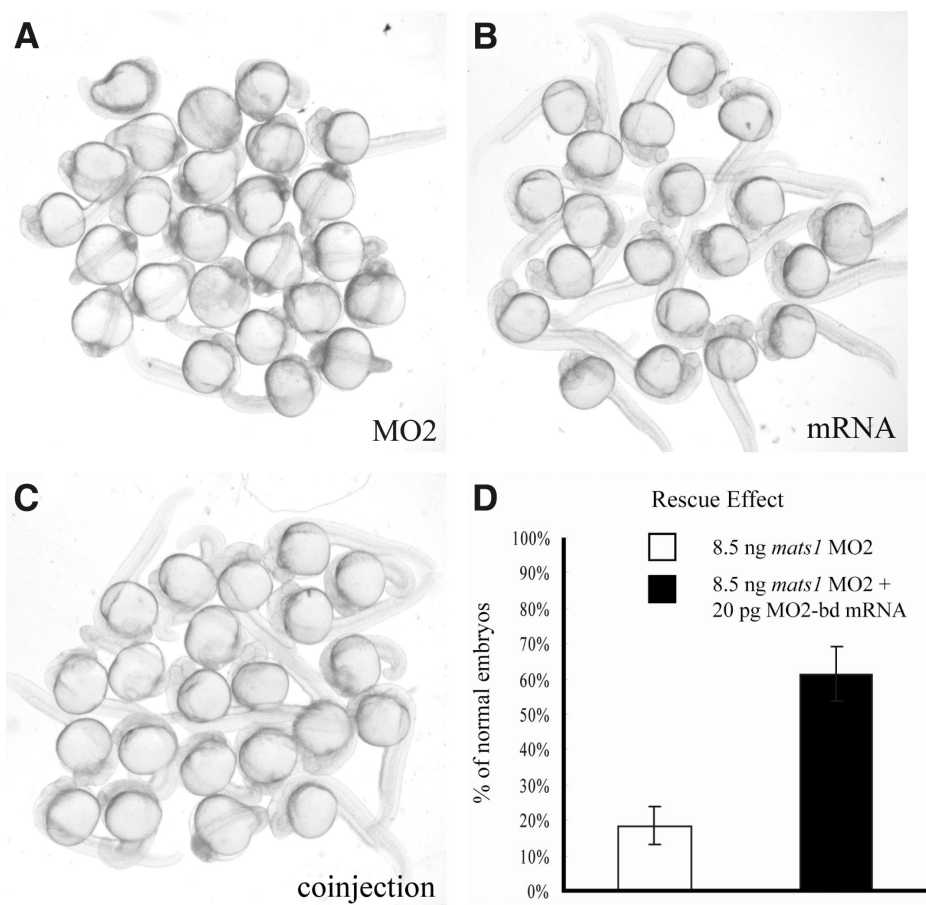
**B**



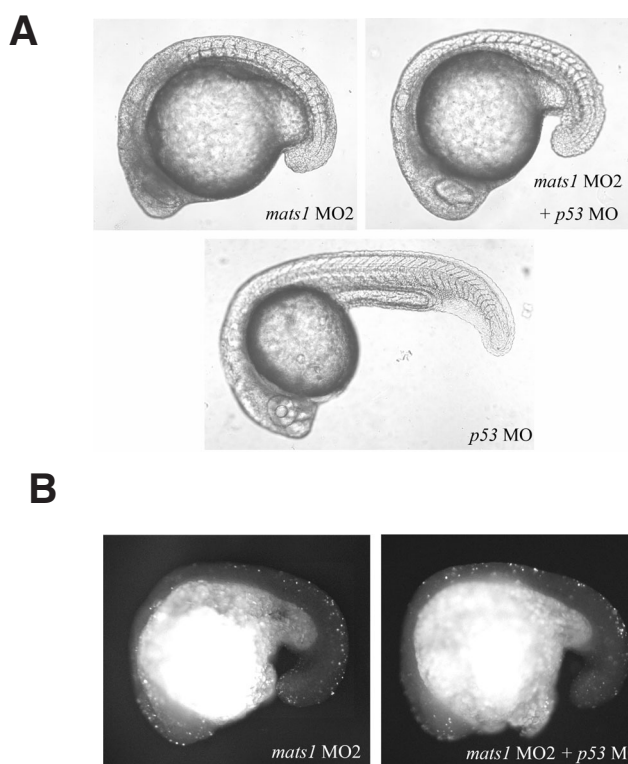
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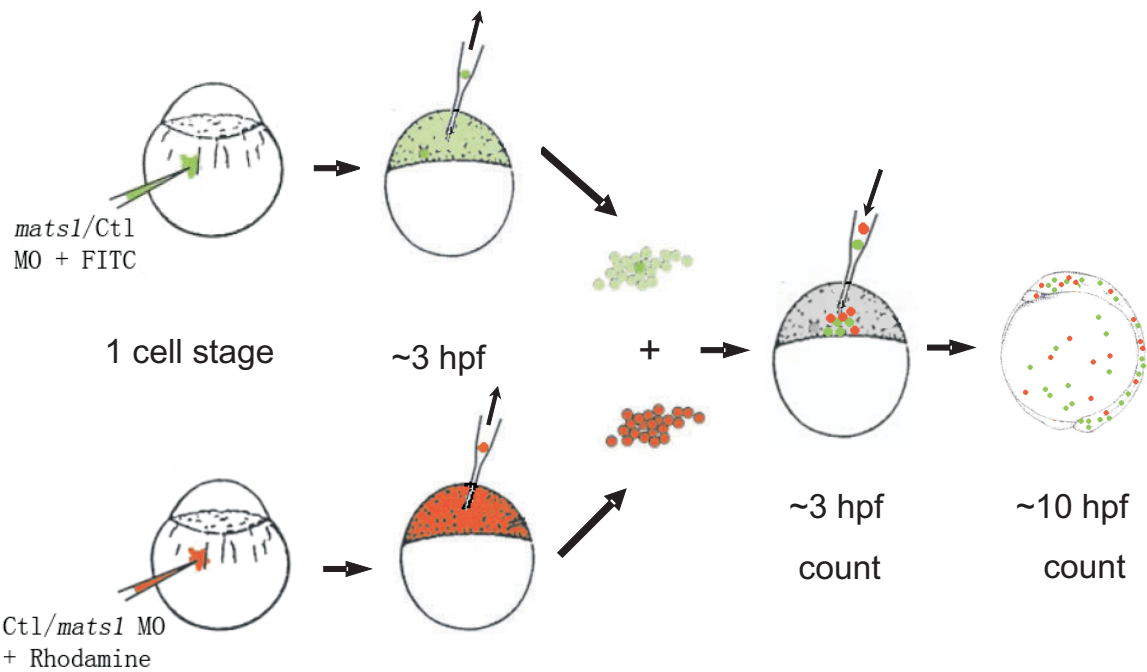
**Fig. S1. Comparison of mats genes from Drosophila, zebrafish, mouse and human.** (A) Sequence alignment of Mats proteins from *Drosophila melanogaster*, *Danio rerio* and *Homo sapiens*. Identical amino acids are highlighted in black, similar amino acids are in grey, and different amino acids with a white background. (B, C) Synteny analysis of flanking regions of *mats1* (B) and *mats2* (C) genes from human, mouse and zebrafish. The chromosomal locations of genes are not in scale.



**Fig. S2. Rescue of *mats1* MO2-induced developmental delay phenotype by MO2-bd *mats1* mRNA.** (A) Embryos injected with 8.5 ng *mats1* MO2. (B) Embryos injected with 20 pg MO2-bd mRNA exhibited normal phenotype. (C) Most embryos co-injected with 20 pg MO2-bd mRNA and 8.5 ng *mats1* MO2 showed normal or less severe abnormal phenotype. (D) Statistical analysis of rescue results. At 24 hpf, only 18% ( $n=182$ ) of the *mats1* MO2 morphant embryos were normal. However, co-injection of 20 pg MO2-bd mRNA with *mats1* MO2 made 61% ( $n=150$ ) of the embryos to become normal ( $T$ -test,  $p < 0.05$ ).



**Fig. S3. Phenotypes of *mats1* morphants were not caused by off-target *p53* activation.** (A) The *mats1*/*p53* morphant embryos still exhibited the developmental delay phenotype. Embryos injected with 17 ng *p53* MO didn't show any abnormal phenotype. When coinjected with 17 ng *p53* MO plus 8.5 ng *mats1* MO2, the severe developmental delay phenotype still exist, but the small, dark, necrotic heads phenotype was lessened. (B) The *mats1*/*p53* double knockdown embryos still exhibited excessive apoptosis as demonstrated by TUNEL staining, with decreased signal in head region. Anterior is towards left and dorsal towards top in all panels.



**Fig. S4. Transplantation of labeled *mats1* morphant cells and wild-type cells to generate chimeric zebrafish embryos.** Schematic illustration of the transplantation experiment is shown. *mats1* MO2 mixed with a dye (e.g. FITC) was injected into embryos at the 1-cell stage. The control MO mixed with a different dye (e.g. Rhodamine) was similarly injected. At the 3-4 hpf stage, FITC-labeled *mats1* morphant cells and Rhodamine-labeled control cells were collected and mixed before transferring to wild-type embryos at the 3-4 hpf stage. Cell number was counted under a fluorescent microscope when transplantation was done, and counted again at the 10 hpf stage. Cell number at 10 hpf divided by cell number at 3-4 hpf is defined as proliferation index (PI) to show cell proliferation ability. In another experiment, *mats1* morphant cells labeled with Rhodamine were also tested for their ability to proliferate compared to FITC-labeled control cells. For additional control experiments, cells containing only FITC or Rhodamine were also tested in this assay.