SUPPLEMENTARY MATERIAL

corresponding to:

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

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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 co-injected 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.