THE INTERNATIONAL JOURNAL OF
DEVELOPMENTAL
BIOLOGY
www.intjdevbiol.com

doi: 10.1387/ijdb.130065xs

SUPPLEMENTARY MATERIAL

corresponding to:

Zfyve9a regulates the proliferation of hepatic cells during zebrafish embryogenesis

NIAN LIU, ZHUO LI, DUANQING PEI and XIAODONG SHU

***Address correspondence to:** Xiaodong Shu. Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, 190 KaiYuan Avenue, Science Park, Guangzhou 510530, China. Fax: +86-20-3201-5231. Tel: +86-20-3201-5292. E-mail: shu_xiaodong@gibh.ac.cn

Full text corresponding to this paper is available at: http://dx.doi.org/10.1387/ijdb.130065xs

Supplementary Methods

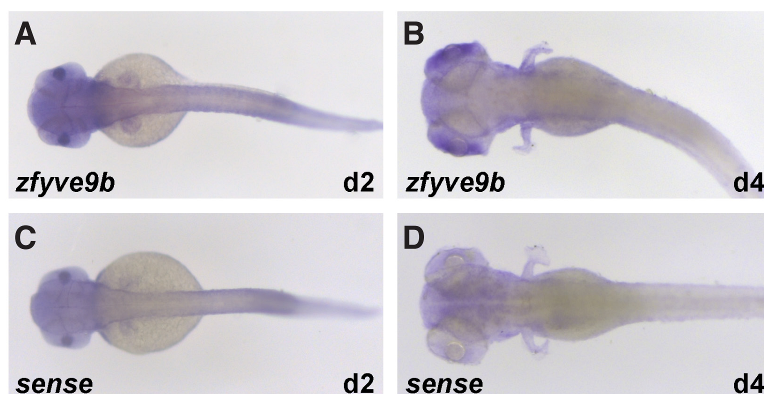
Western blot

Embryos were injected with mRNA encoding 3*Flag tagged human SMAD2 (120 pg) together with MO-AUG or a control morpholino (MO-CTL, 4 ng/embryo). Alternatively, Embryos were injected with mRNA encoding 3*Flag tagged human SMAD2 (120 pg), and at 2-cell stage, TGF-β pathway inhibitors (Repsox (Sigma Aldrich, 10 μM) or DMSO (Sigma Aldrich, 0.5%)) were added to the samples.

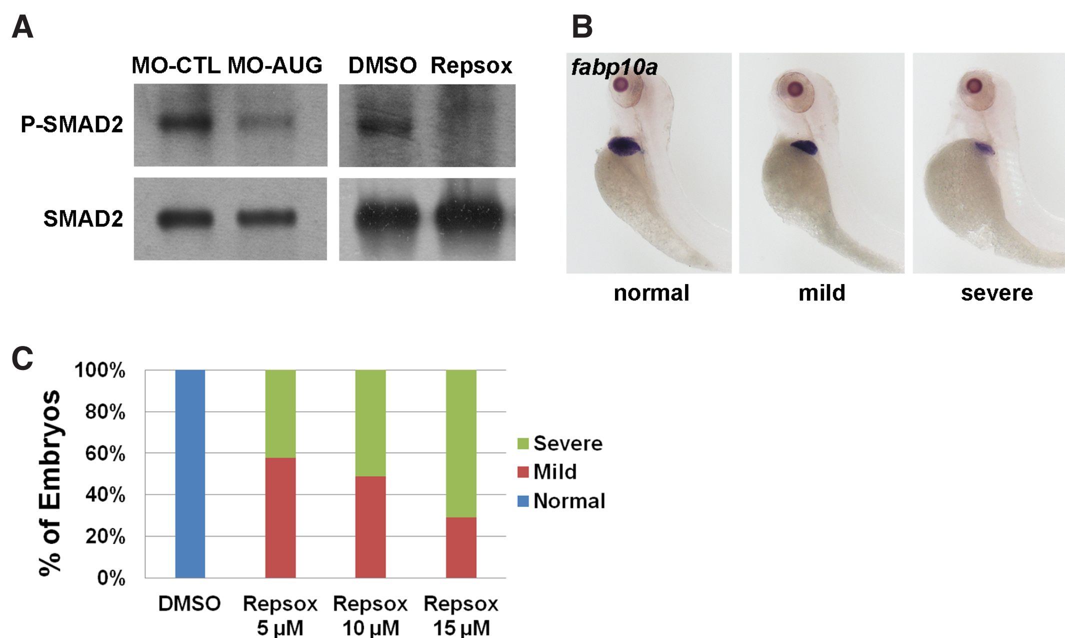
Fish embryos were harvested at 50% epiboly stage and deyolked by passing the embryos through the 50 microliter eppendorf pipette tip in cold Ringer's solution with EDTA and PMSF for several times. The embryos were then shaken for 5 min at 1100 rpm to dissolve the yolk (Thermo-mixer, Eppendorf). Deyolked embryoes were pelleted at 300 g for 30 sec and the supernatant discarded. Then RIPA lysis buffer (Cell Signaling, MA) were added to the deyolked embryos, followed by ultrasonication. Finally, SDS sample buffer was added to the ultrasonicaed embryos and samples were boiled for 5 min, cleared by centrifugation at 12,000 rpm for 5 min and the supernatants separated by SDS-PAGE. Western blot was performed according to standard protocol. The following antibodies were used: phospho-smad2 (ser465/467) antibody (Cell Signaling, 1:1000), monoclonal anti-FLAG® M2 antibody (Sigma, 1:5000).

TGF-β inhibitor treatment

Embryos were raised in 6-well plates (30 embryos/5 ml E3 solution) up to 24 hpf and TGF-β pathway inhibitors (Repsox (Sigma Aldrich, 8-15 μM) or DMSO (Sigma Aldrich, 0.5%)) were added to the samples. Treated embryos were harvested at day 4 for *in situ* hybridization analysis.



Supplementary Fig. S1. The embryonic expression of zebrafish *zfyve9b*. *Embryos were hybridized to an antisense probe to* zfyve9b **(A-B)** *or a sense control probe* **(C-D)**. zfyve9b *was not expressed in endoderm-derived organs at all stages examined.*



Supplementary Fig. S2. The TGF-β signaling is required for liver development in zebrafish. (A) *Treatment of embryos with the MO-AUG or a small chemical inhibitor to the TGFβ receptor (Repsox) reduced the level of P-SMAD2.* **(B)** *Representative results of the Repsox treatment induced liver defect.* **(C)** *Statistical results of (B). Embryos were treated with the indicated dosages of Repsox from day 1 to day 4 and the expression patterns of fabp10a determined by* in situ *hybridization. N=29, 19, 39 and 31 respectively.*