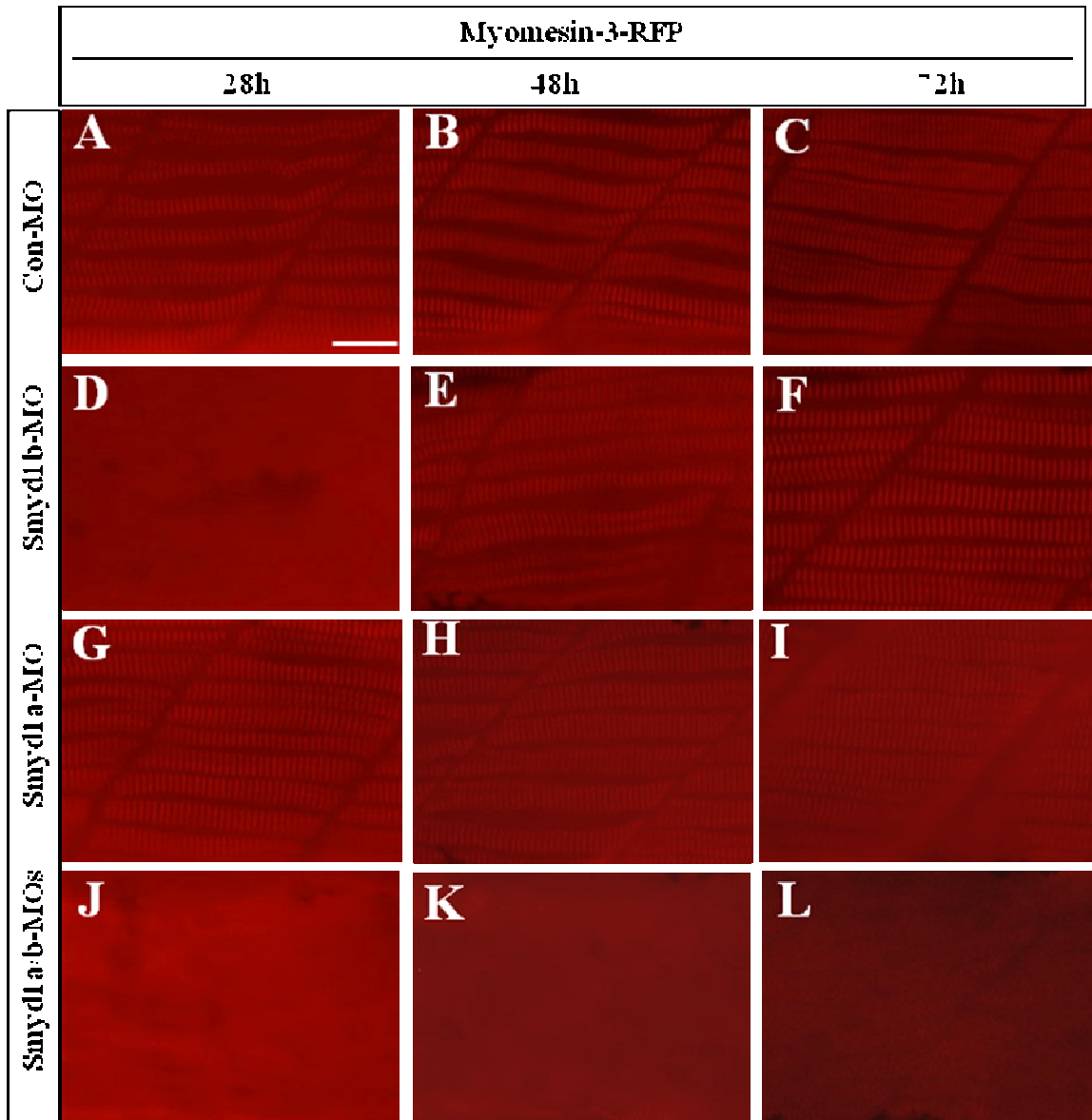


# Supplemental Materials

*Molecular Biology of the Cell*

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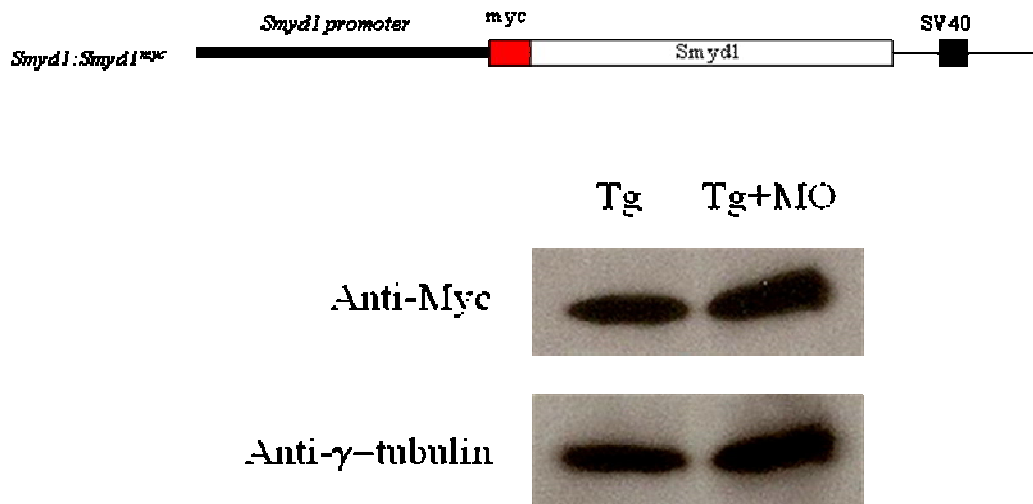
Supplemental material



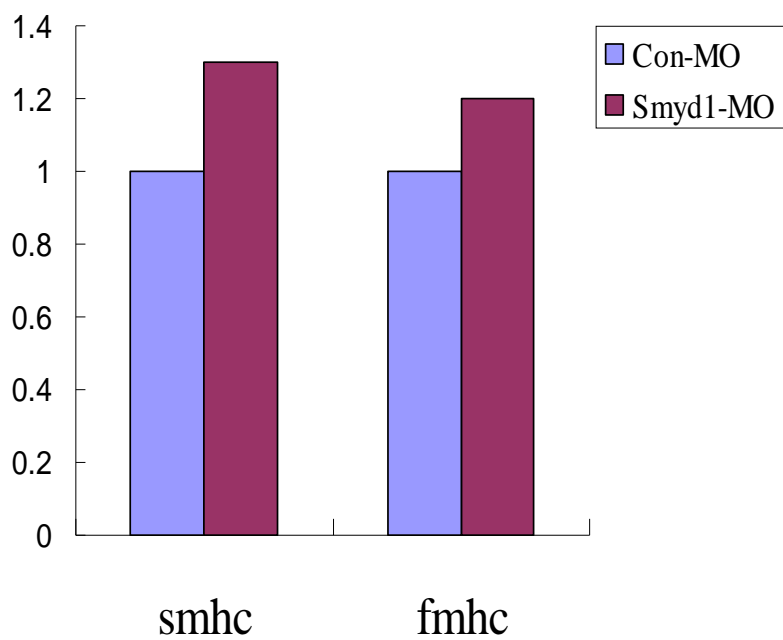
**Fig. 1S. Knockdown of *smyd1b* expression resulted in defective M-line organization in slow muscles of early stage zebrafish embryos.**

Fertilized eggs of myomesin-3-RFP zebrafish were injected with the control-MO (A, B, C), Smyd1b-ATG-MO (D, E, F), Smyd1a-E8I8-MO (G, H, I), or both Smyd1a/b-MOs (J, K, L). The M-line localization of myomesin-3-RFP was analyzed in the injected embryos at 28 (A, D, G, J), 48 (B, E, H, K) and 72 (C, F, I, L) hpf. Compared with the control (A,

B, C), knockdown of Smyd1b disrupted M-line organization in slow muscle of zebrafish embryos at 28 hpf (D), but not at 48 (E) and 72 (F) hpf. Unlike loss of Smyd1b, knockdown of *smyd1a* had no effect on M-line organization of myomesin-3-RFP in all three stages analyzed (G, H, I). However, knockdown of both *smyd1a* and *smyd1b* together disrupted the M-line localization of myomesin-3-RFP at all the stages (J, K, L). Scale bars: 20µm.



**Fig. 2S.** The structure of the *Tg(Smyd1b: Smyd1b<sup>myc</sup>)* transgene and western blot analysis shows the expression of the myc-tagged Smyd1b from the transgene (Tg) in the control (Tg) or Smyd1b E9I9 -MO injected (Tg+MO) embryos at 72 hpf.



**Fig. 3S.** Real-time RT-PCR analysis of SMHC and FMHC mRNA expression in control-MO or Smyd1b-ATG-MO injected embryos at 24 hpf.

**Table 1. Microarray analysis of changes of gene expression profile in smyd1 knockdown embryos.**

Gene expression profiles were compared between control and smyd1 knockdown embryos at 24 hpf. Among the 12 upregulated genes, eight of them encode heat shock protein. Approximately 120 genes were downregulated. Those are expressed in muscles or are involved muscle development were listed in the table.

<p><b>Upregulated genes</b></p>	<ol style="list-style-type: none"> <li>1. <b>Danio rerio HSP70 mRNA for stress protein HSP70 (Hsp70A)</b></li> <li>2. <b>Danio rerio inducible 70 kDa heat shock protein (hsp70) gene (Hsp70B)</b></li> <li>3. <b>Danio rerio Hsp70 gene (Hsp70C)</b></li> <li>4. <b>Danio rerio heat shock protein 90 (hsp90) mRNA</b></li> <li>5. <b>Danio rerio heat shock cognate 70.II</b></li> <li>6. <b>Danio rerio heat shock protein 47</b></li> <li>7. <b>Danio rerio heat shock protein 90-alpha 1</b></li> <li>8. <b>Danio rerio heat shock cognate 70</b></li> </ol>
<p><b>Downregulated genes</b></p>	<ol style="list-style-type: none"> <li>1. <b>Danio rerio DMbeta2a mRNA</b></li> <li>2. <b>Danio rerio mesoderm posterior a (mespa)</b></li> <li>3. <b>Danio rerio clone VH88 immunoglobulin heavy chain variable region</b></li> <li>4. <b>Danio rerio ets related protein erm (erm)</b></li> <li>5. <b>Danio rerio homeobox protein (hoxb10a) gene</b></li> <li>6. <b>Danio rerio eukaryotic translation initiation factor 4e 1b(eif4e1b)</b></li> <li>7. <b>Danio rerio SRY-box containing gene 21a (sox21a)</b></li> <li>8. <b>Danio rerio Cecr1 (cecr1)</b></li> <li>9. <b>Danio rerio zinc finger homeobox 1 (zfhx1)</b></li> <li>10. <b>Danio rerio translocon-associated protein beta</b></li> <li>11. <b>Danio rerio L-plastin</b></li> <li>12. <b>Similar to Homo sapiens mRNA for alpha actinin 4</b></li> <li>13. <b>Similar to Fugu rubripes beta-cytoplasmic actin2 gene</b></li> <li>14. <b>Similar to Homo sapiens TANK binding kinase TBK1 (TBK1)</b></li> <li>15. <b>Similar to Homo sapiens deubiquitinating enzyme UnpES (UNP)</b></li> <li>16. <b>Similar to Homo sapiens calbindin 2, 29kDa (calretinin)</b></li> <li>17. <b>Smimilar to Oryctolagus cuniculus mRNA for calmodulin-dependent protein kinase</b></li> <li>18. <b>Similar to Sus scrofa CYP51 gene for lanosterol 14 alpha-demethylase</b></li> </ol>