# Development of teleost intermuscular bones undergoing intramembranous ossification based on histological-transcriptomic-proteomic data

**Chun-Hong Nie [1,2], Shi-Ming Wan [1,2], Yu-Long Liu [4], Han Liu [1,2], Wei-Min Wang [1,2] and Ze-Xia Gao [1,2,3*]**

[1] College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education/Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei 430070, China; 1508085740@qq.com (C.H. N); 276222007@qq.com (S.M. W); 592299190@qq.com (H. L); wangwm5911@gmail.com (W.M. W)

[2] Engineering Technology Research Center for Fish Breeding and Culture in Hubei Province, Wuhan 430070, China

[3] National Demonstration Center for Experimental Aquaculture Education, Huazhong Agricultural University, Wuhan 430070, China

[4] Department of Molecular and Cellular Biology, University of California Davis, Davis 95616, California, USA; idrliu@ucdavis.edu (Y.L. L)

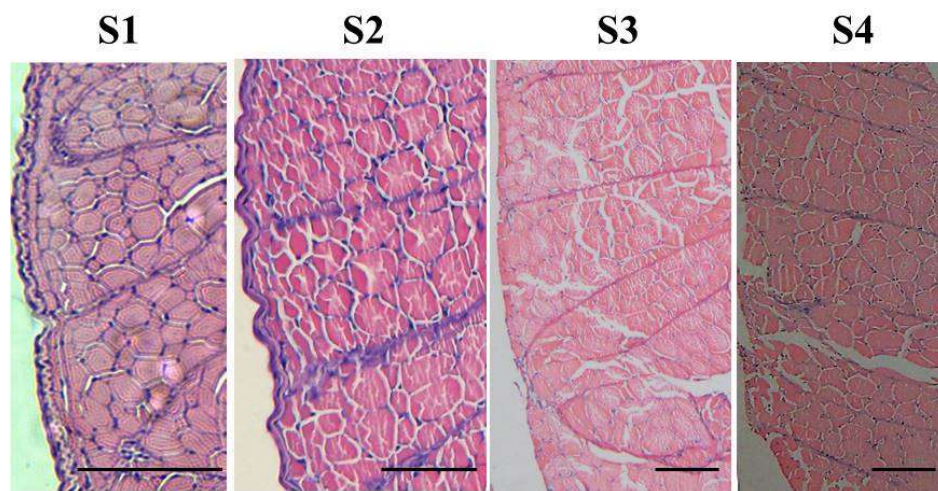* Correspondence: gaozexia@hotmail.com; Tel/Fax: +86-27-8728-2113

**Figure S1** The histology structure with HE staining of muscle tissues at four stages. Scales bars = 50 μm.
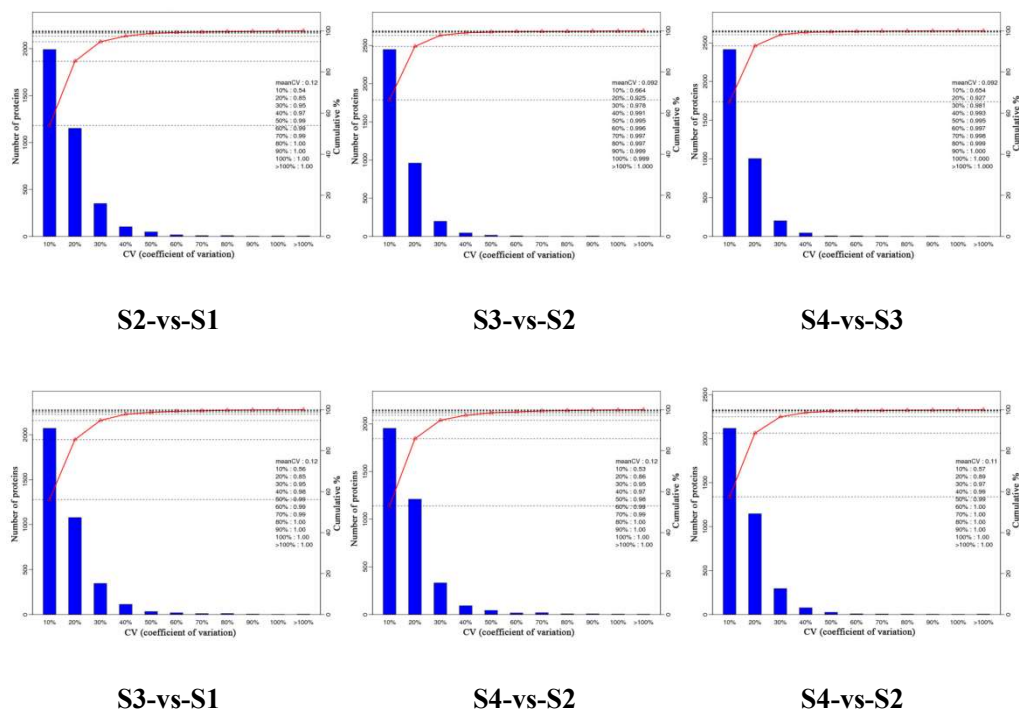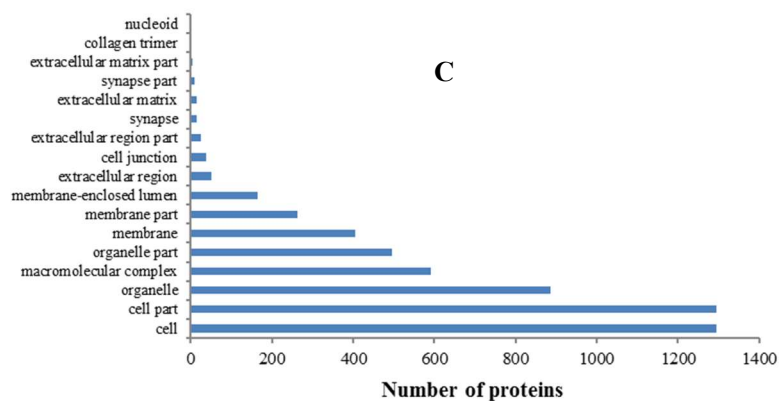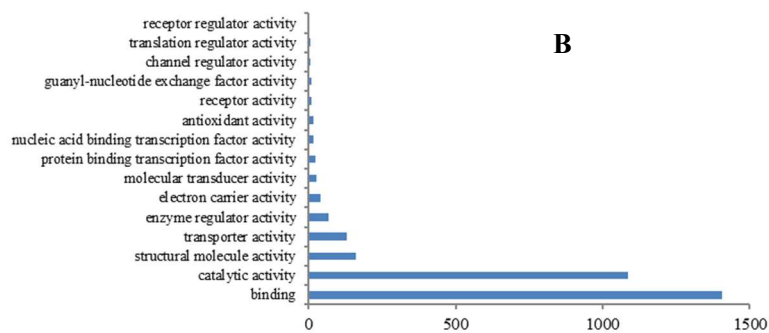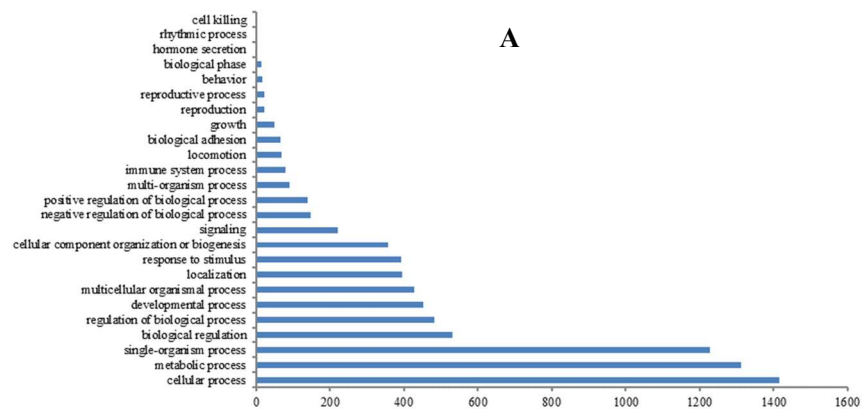
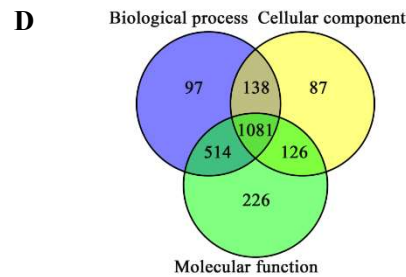**Figure S2** Repeatability analysis of data obtained from iTRAQ in different groups.

Number of proteins

**D** Biological process  Cellular component

97 138 87

1081

514 126

226

Molecular function

**Figure S3** Proteins were functionally annotated for (**A**) biological process, (**B**) molecular function, as well as (**C**) cellular component and Venn diagram of proteins annotated for three processes (**D**).
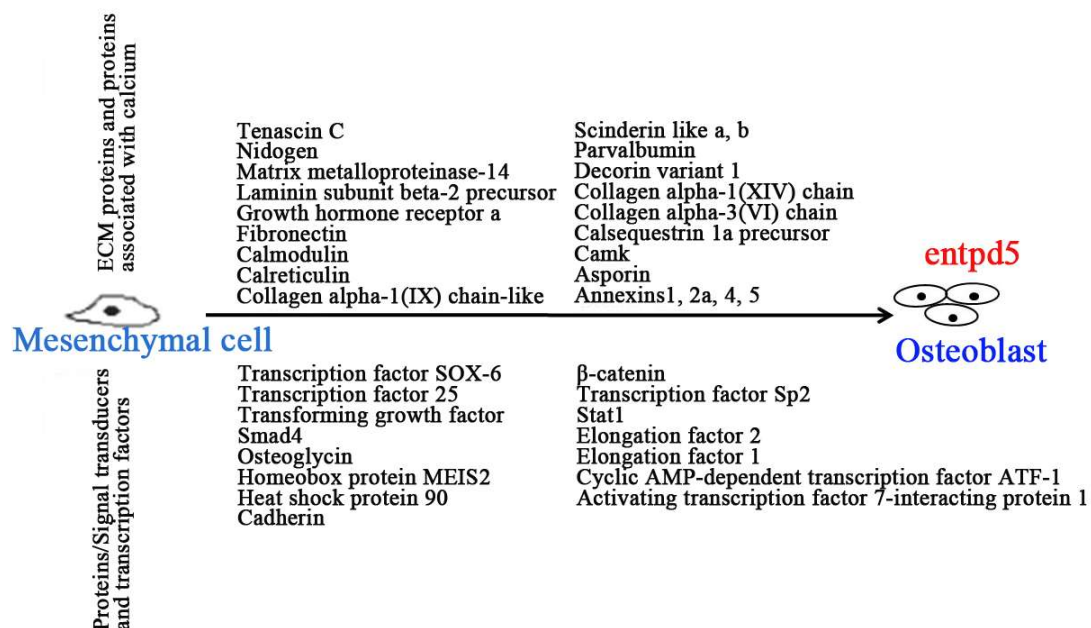


**Figure S4** Schematic representation of the key proteins in osteoblastogenesis during IB ossification process.
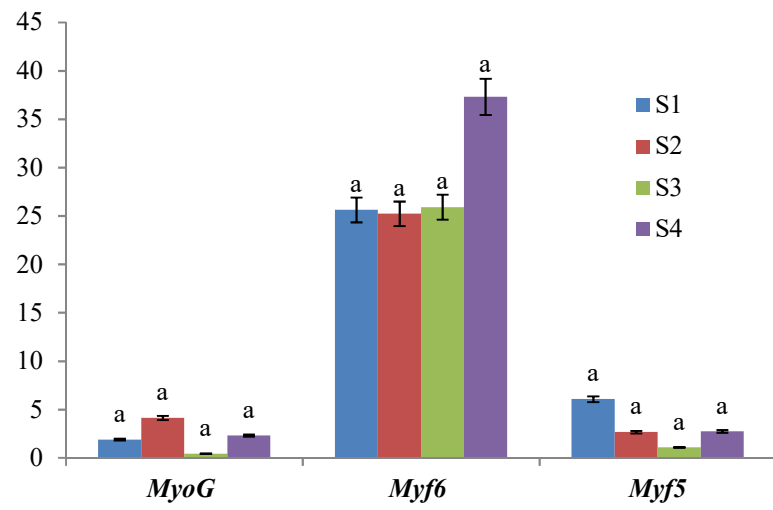
**Figure S5** Expression of muscle-related genes at different stages based on RNA-seq data.
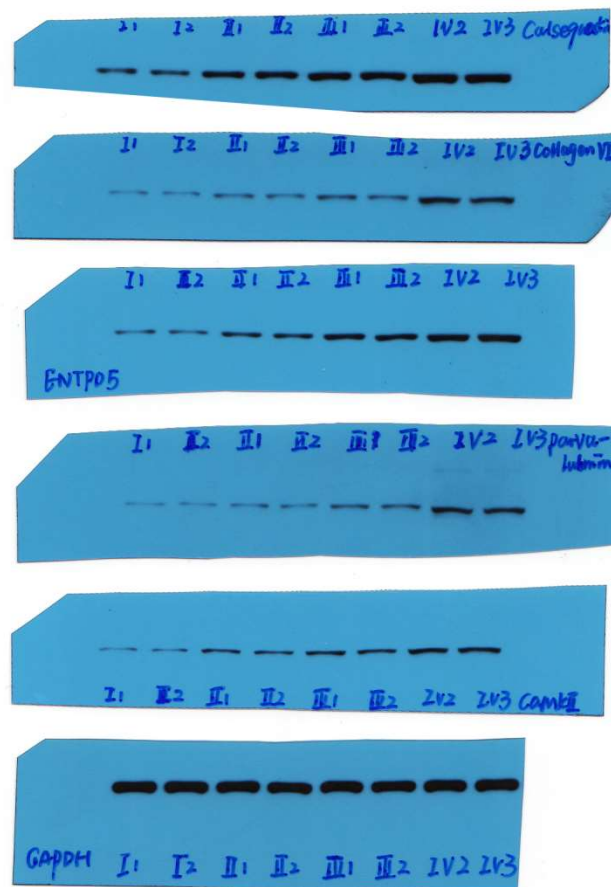
**Figure S6** The raw data photo of western blot for five proteins; GAPDH is used as a loading control.
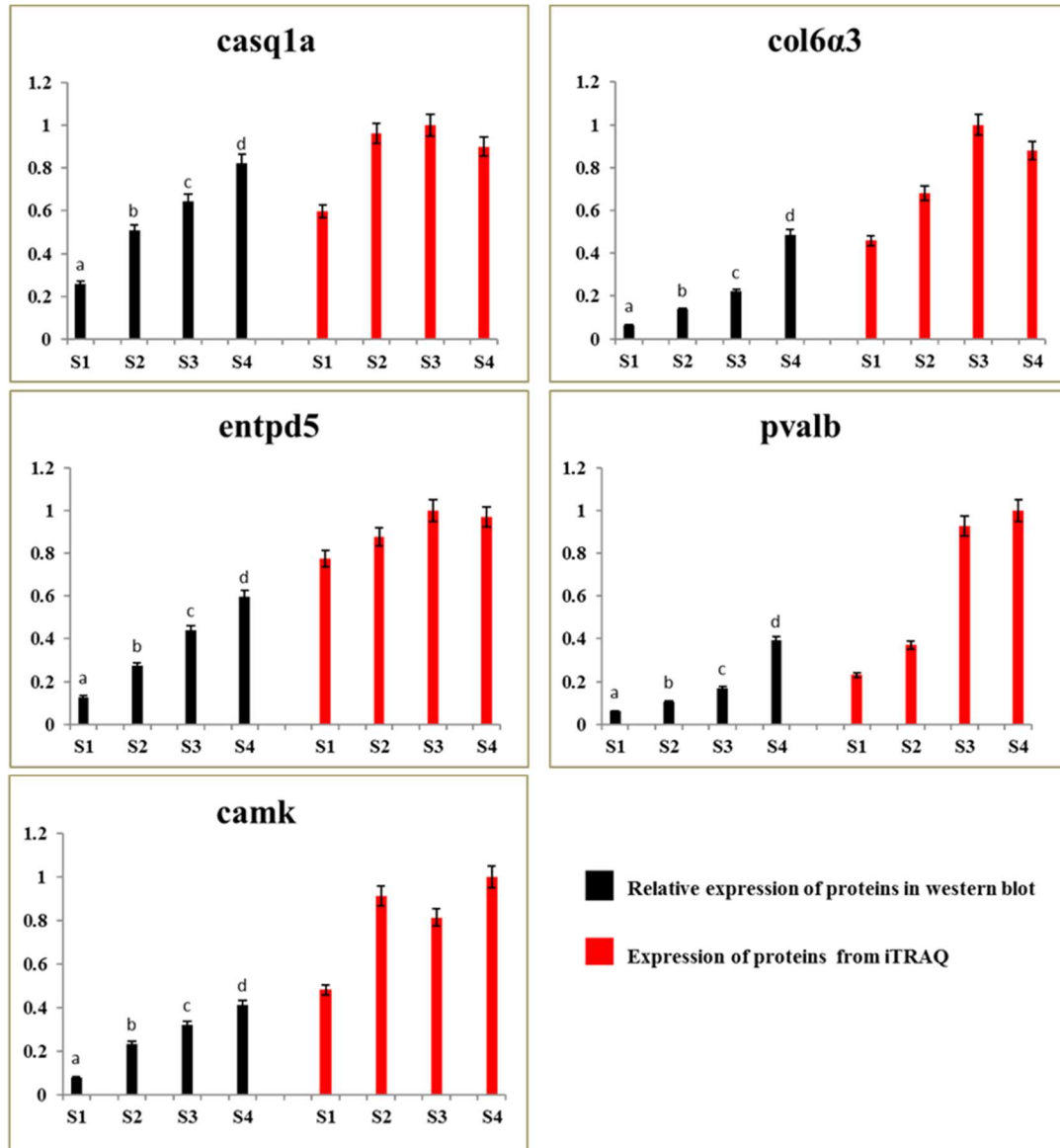
**Figure S7** Comparison of expression levels for the 5 detected bone-related proteins based on iTRAQ and western blot results. Different letters above pillars for each stage indicated statistical significance at the level of $p < 0.05$.
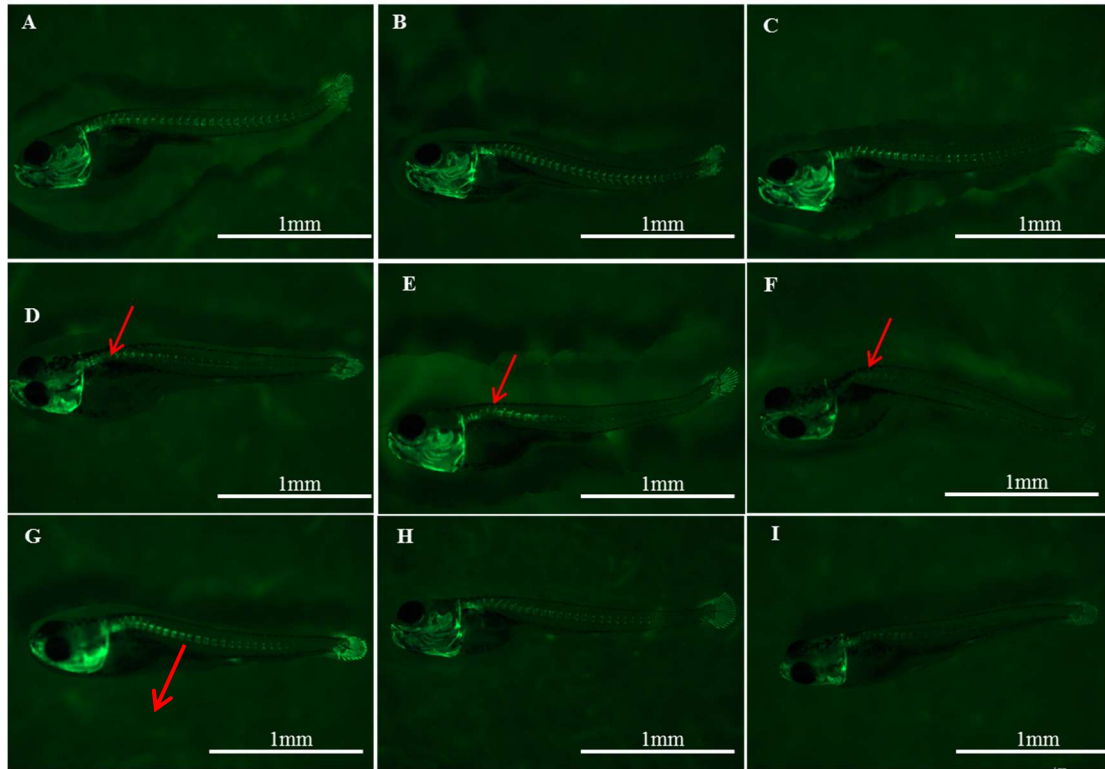
**Figure S8** Effects of IWP-L6 (D–F) and K02288 (G–I) on Tg (*sp7/eGFP*) expression of vertebrate in zebrafish larvae. Control larvae (A, B, C) were treated with DMSO. Red arrows represent that part of vertebrate had a curved phenomenon.