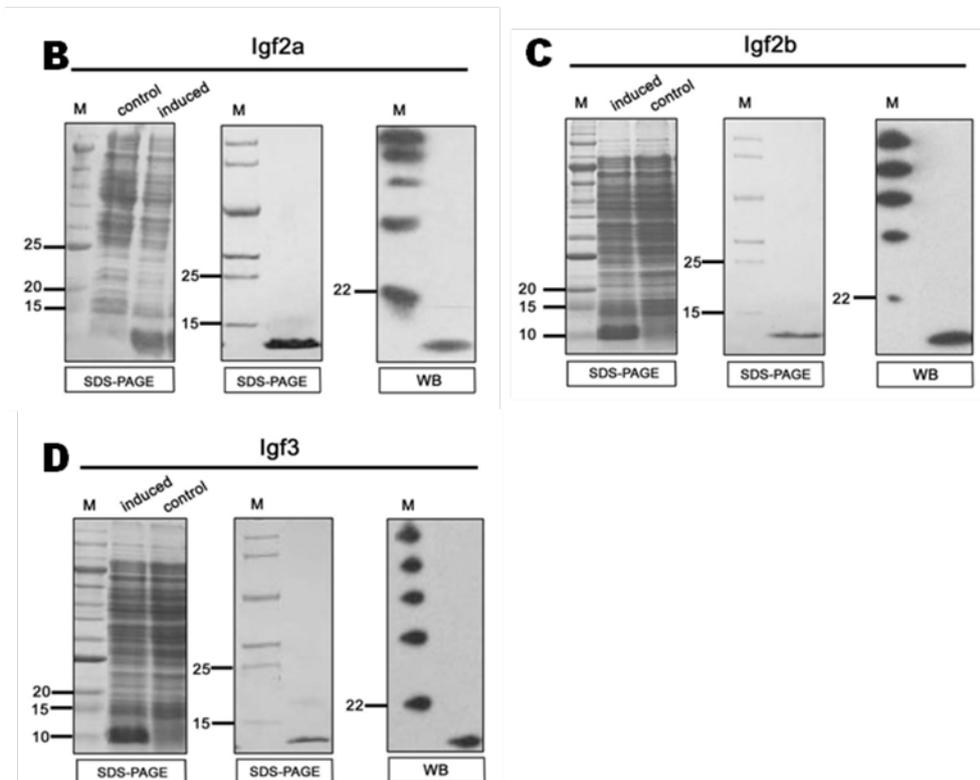


Supplemental Figure 1. Targeted disruption of the zebrafish *lhb* gene by TALENs.

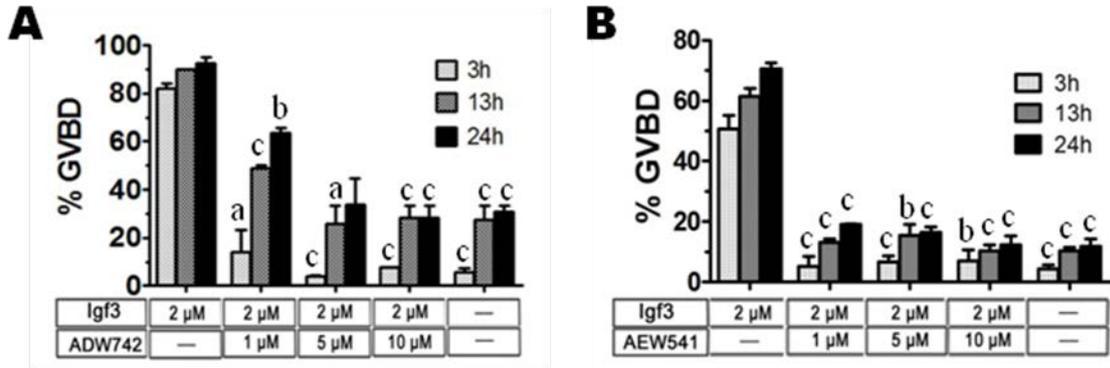
A. A flow diagram indicating the establishment of *lhb* knockout zebrafish. B. The location of the engineered TALENs binding sites in the *lhb* gene of zebrafish. The sequences of the pair of TALENs binding sites are underlined. WT, wild-type; M, homozygous mutant line of F2 generation zebrafish; +, inserted nucleic acids number; Δ, deleted nucleic acids number. (Chu *et al.*, 2014) (15).

## A

Igf2a GETLCGGELVDTLQFVCGEDGFYISRPNRSNSRRPQRGIVEECCFRSCELHLLQYCAKPVKSER  
Igf2b SAETLCGGELVDALQFVCEDRGFYFSRPTSRSNSRRSQNRGIVEECCFSSCNLALLEQYCAKPAKSER  
Igf3 ARARCGRELVDLLEFVCGDRGFYIGKPGAARSGGPRSRGKGIVDQCCVRGCDLQHLLELYCAKSKKVRRD

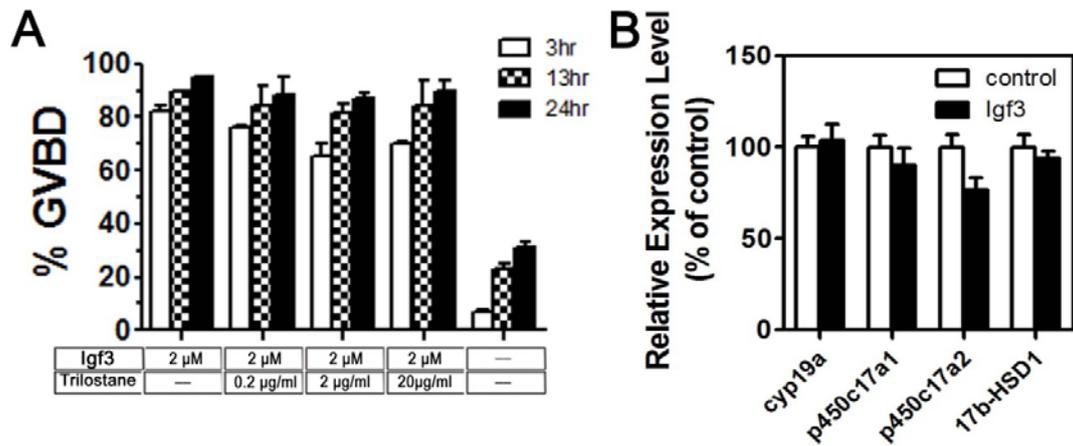


Supplemental Figure 2. Preparation of recombinant zebrafish Igf2a, Igf2b and Igf3 proteins. (A) Amino acid sequences of mature zebrafish Igf2a, Igf2b and Igf3. Recombinant Igf2a (B), Igf2b (C) and Igf3 (D) proteins were prepared in an *E. coli* expression system. The results were analyzed by SDS-PAGE, followed by commassie blue staining. A strong band could be seen in the IPTG induction group.



Supplemental Figure 3. Effects of Igf1r inhibitors on stimulation of Igf3 oocyte maturation in zebrafish.

(A) Inhibition of Igf3-induced oocyte maturation by different doses of NVP-AEW541. (B) Inhibition of Igf3-induced oocyte maturation by different doses of NVP-ADW742. Each value represents the mean value  $\pm$  SEM of triplicate assays from three independent experiments (a,  $P < 0.05$ ; b,  $P < 0.01$ ; c,  $P < 0.001$  vs. control).



Supplemental Figure 4: Effects of steroidogenesis inhibitor on Igf3-induced oocyte maturation and effect of Igf3 on expression of steroidogenesis enzymes.

(A) The lack of effect of trilostane (a  $3\beta$ -HSD inhibitor) on Igf3-induced oocyte maturation at different concentrations (0.2, 2 and 20  $\mu$ g/ml). FG stage follicles were treated with recombinant Igf3 (2  $\mu$ M) in the absence or presence of trilostane, and GVBD was scored after the treatment. (B) Relative mRNA level of *cyp19a*, *p450c17a1*, *p450c17a2* and *17b-HSD1* in MV stage follicles treated with zebrafish Igf3 protein (2 $\mu$ M) for 1 h. White bar, control; Black bar, Igf3 treatment. Each value represents the mean  $\pm$  SEM of three independent experiments each performed in quadruplicates.