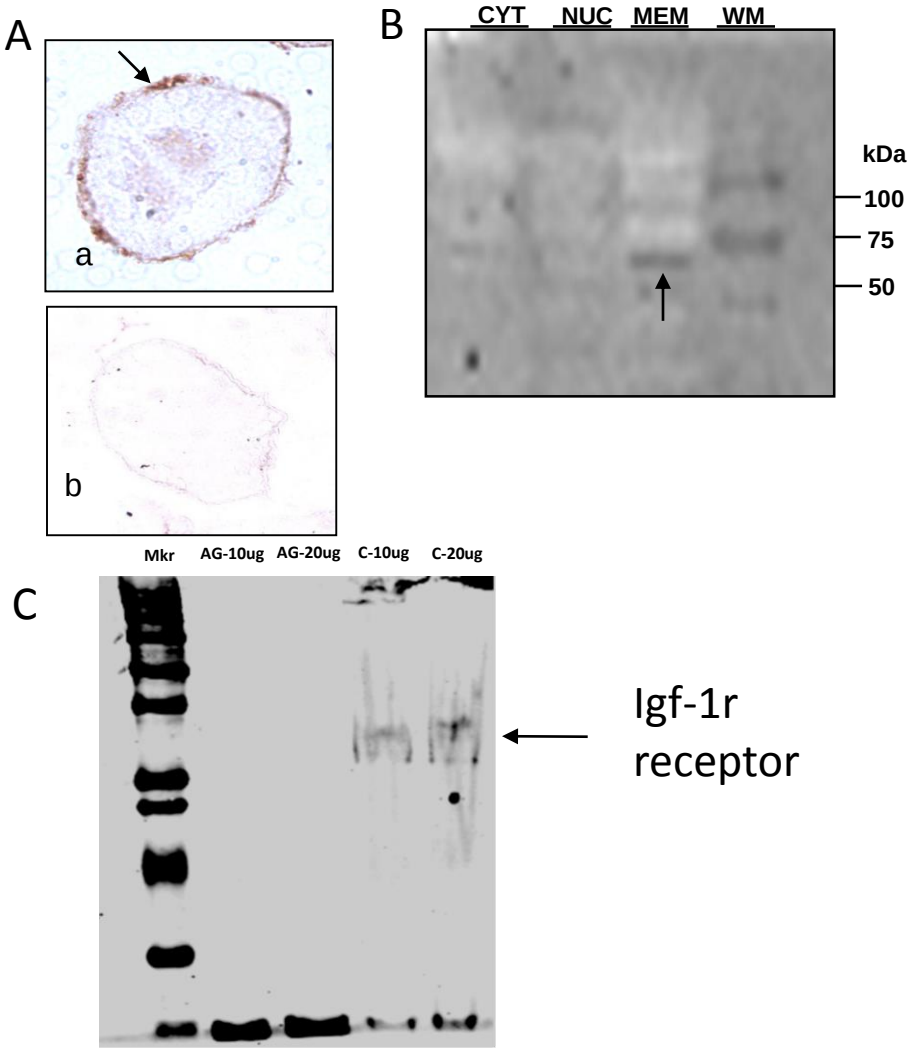
Supplementary Fig. 3



Supplementary Figure 3. Detection of Igf-1 receptor on zebrafish oocyte membranes. (A) a, immunodection of Igf receptor on plasma membranes of denuded zebrafish oocytes (arrow); b, negative control without 2ndy antibody. Ovarian sections were deparaffinized in xylene, rehydrated, blocked with 1% BSA and incubated with rabbit human IGF-R polyclonal antibody raised in rabbits to the middle region of HIGF-R (Avia Systems Biology # AVARp00004_P050), dilution 1:100. The slides were washed in PBS after then were incubated with a fluorescent dye-conjugated goat anti-rabbit IgG HRP-linked secondary antibody (dilution 1:100, Southern Biotech, Birmingham, AL) at room temperature for 4hrs. The slides were washed again and incubated with Immpact DAB (Vector labs, #SK4105) for color development.

Supplementary Fig. 3

(B) Western blot of subcellular fractions of zebrafish oocytes showing a band of the predicted size (~ 70 kDa) for Igf-1r in plasma membrane fraction; (C) Western blot of Igf-1r in zebrafish oocyte membranes after treatment with AG205. Western blot analysis was carried out on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel with 24 µg of protein per lane, transferred to nitrocellulose membranes, blocked with dry milk, PBS, and 1% Tween-20 for one hour. Membranes were then incubated overnight with the human IGF-R antibody (Avia Systems Biology Corp, #AVARp0004_P050) at a dilution of 1:1000. The membranes were incubated with a goat anti-rabbit IgG HRP-linked secondary antibody , treated with enhanced luminescence reagent and detected on the Odyssey Infrared Imaging System at 800nm.