Method for immunofluorescence: Fix the cells by incubating in 4% (w/v) paraformaldehyde for 10 min at room temperature. Rinse the cells three times and permeabilized the cells in PBS containing 0.1% Triton X-100 for 10 min at room temperature (RT). These cells were incubated in 2% bovine serum albumin for 30 minutes and then with PAX7 (Santa Cruz Biotechnology, Santa Cruz, CA), MYOD1 (Santa Cruz Biotechnology, Santa Cruz, CA), and TSH receptor monoclonal antibodies (Invitrogen, Carlsbad, CA) for 2 hours. Each staining was performed separately. After washing with PBS, cells with PAX7 were incubated with fluorescent anti-mouth antibody (Alexa 488; Thermo Fisher Scientific), cells with MYOD1 were incubated with fluorescent anti-mouse IgG antibody (Alexa 594; Thermo Fisher Scientific), and cell with TSH receptor antibodies were incubated with anti-mouse antibody (Alexa 488; Abcam, Cambridge, UK) for 1 hour, respectively. Nuclei were stained by DAPI (Vector Laboratories Inc., Burlingame, CA) for 2 minutes. All these cell images were analyzed using Axio imager M1 microscope (Carl ZEISS, Oberkochen, Germany).

Method for reverse transcription-polymerase chain reaction (RT-PCR): Total RNA was extracted from human extraocular muscle myoblasts at 0 day, using QIAzol^{TM} Lysis Reagent (Qiagen, USA). Samples were lysed in the QIAzol^{TM} Lysis Reagent and chloroform was added. The mixture was centrifuged at 12,000 rpm at 4°C for 15 min. The RNA pellets were washed with 75% ethanol by vortexing, and then the pellets were dried briefly and dissolved in diethypyrocarbonate (DEPC)-treated RNase-free solution. Purified RNA was incubated with the DNase I Solution for 15 min at 37°C, followed by 65°C for 20 min. Total RNA concentration was determined by measuring absorbance at 260 nm. Prepared RNA was reverse transcribed into complementary DNA (cDNA) with reaction buffers. The reaction mixture was incubated at 50°C for 50 min, followed by 70°C for 10 min. The PCR primer pairs for cDNA amplification were as follows: human *SHOX2* (sense) 5′-

GACGAGACCCACTATCCCGA -3′ and (antisense) 5′-CTACATTTAGCTCTTCGATTTTGA-3′; human *HOXC10* (sense) 5′-GACCCCCAGCCCCAATGAAA-3′ and (antisense) 5′-TTCTTTCTCCAATTCCAGCGTCT-3′; and human *GAPDH* (sense) 5′-GGGGAGCCAAAAGGGTCATCATCT-3′ and (antisense) 5′- GAGGGGCCATCCACAGTCTTCT-3′ Each PCR sample was electrophoresed on a polyacrylamide gel. The gels were dried and subjected to autoradiography using Kodak X-ray films to show the amplified DNA products.

Method for Western blot: Equal amounts of protein (30 µg) from each sample were separated on 10% SDS-polyacrylamide gels, then transferred to nitrocellulose membranes. The membrane was subsequently blocked with 5% bovine serum albumin in tris-buffered saline containing 0.1% Tween-20 for 1 h at room temperature, after which it was incubated overnight at 4°C with TSH receptor binding protein (Invitrogen, Carlsbad, CA) primary antibodies. Next, the membrane was incubated with secondary antibody at room temperature for 2 h. Bands were subsequently detected using an enhanced chemiluminescence detection kit (Thermo Scientific), after which these blots were re-probed with rabbit monoclonal anti-β-actin antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a loading control for all experiments.