Oil Red O (ORO) Staining

For the histological examination, adult males and females were sacrificed and livers (N=3) were dissected and fixed with 4% paraformaldehyde in PBS (pH 7.4). After washing with PBS, livers were immersed in 30% sucrose solution at 4°C. Subsequently, livers were embedded with OCT resin (Killik, Bio Optica) and stored at -80°C until the sectioning. Specimens of all experimental groups were serial sectioned (10 µm). Liver cryosections were incubated in 60% isopropanol (5 min) and stained in Oil Red O solution (0.5% isopropanol) for 30 minutes. After 5 min in 60% isopropanol, sections were rinsed twice in distilled water, mounted in glycerol and imaged using Zeiss AxioImage M1 microscope.

Western Blot analysis

Zebrafish livers (N=3/each experimental group) were dissolved in RIPA buffer [50 mM Tris (pH 7.4)], 150 mM NaCl, 0.1% SDS, 0.5% Na-deoxycholate, Nonidet P-40, protease and phosphatase inhibitor mixture (Sigma)] (200 µL/liver), homogenized and incubated on ice for 20 min. After full speed centrifugation for 20 min at 4°C to remove insoluble particles, the supernatants were collected and quantified. 10% SDS-gels were run, blotted onto nitrocellulose membrane, and blocked 1 h in blocking buffer (5% non-fat dry milk in TBS containing 0.5% Tween-20). Primary antibodies were incubated overnight at 4°C in blocking buffer. Primary antibodies used were: P-Foxo3a (S253, 1:1000) and Foxo3a (75D8, 1:1000) (Cell signalling), and SREBP1c (28481, 1:1000) (Abcam). After four washes for 30 min in TBST, the membrane were incubated 1 h with secondary antibody in 2% non-fat dry milk in TBST (1:2000). To normalize the data, mouse B-actin (1:3000) (Cell Signalling) were used.

Immunohistochemical staining of thyroid follicles.

For whole-mount antibody staining, larvae at 7 dpf were fixed in paraformaldehyde (PFA, 4%) at 4°C overnight, washed in phosphate-buffered saline containing 0.1% Triton X-100 (PBST), and stored in methanol at –20°C. To bleach and to block endogenous peroxidases, larvae were incubated in 10% H2O2 for 30 min and then blocked in 10% goat serum for 2 h before addition of rabbit anti-T4 (1:4000, MP Biochemicals) and anti-human thyroglobulin (1:6000, Dako) overnight at 4°C. After three washes for 20 min in PBST, larvae were incubated with biotinylated antibody diluted 1:1000 (ABC Vectastain) for 2 h at room temperature. To remove excess antibody, larvae were washed again and incubated with AB reagent following manufacturer’s instructions. After several washes with PBST, thyroid follicles were visualized using diaminobenzidine solution (Vectastain). For detailed analysis, larvae were post-fixed in 4% PFA for 15 min at room temperature, washed in PBST, and gradually transferred to 70% glycerol. Samples were imaged on Zeiss AxioImager M1 microscope with 20x and 40x objectives.