Supplemental Fig. 3. Western blot analysis of the DHH protein in +/+ and mp/mp testes. Testes of +/+ and mp/mp rats at 35 days of age were homogenized in lysis Buffer (Dulbecco’s PBS containing 0.5% CHAPS and 1 × Protease Inhibitor), frozen and thawed three times, and centrifuged at 19000 × g for 30 min. The proteins (20μg) were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membrane was incubated with anti-mouse DHH goat IgGs (AF196; R&D Systems, Minneapolis, USA and M-20; Santa Cruz Biotechnology, Santa Cruz, USA) and anti-beta Actin (ab16039, Abcam, Cambridge, UK) overnight and visualized. The results indicated that a positive band of approximately 44 kDa (arrow) which concordant with predicted molecular mass of the DHH precursor protein appeared in the +/+ testis, but the band was not detected in the mp/mp testis. The same result was obtained by using the two different antibodies to DHH, therefore this 44 kDa band was regarded as the DHH protein. Western blotting using anti-beta actin was also shown (ACTB).