Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis

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Supplementary information

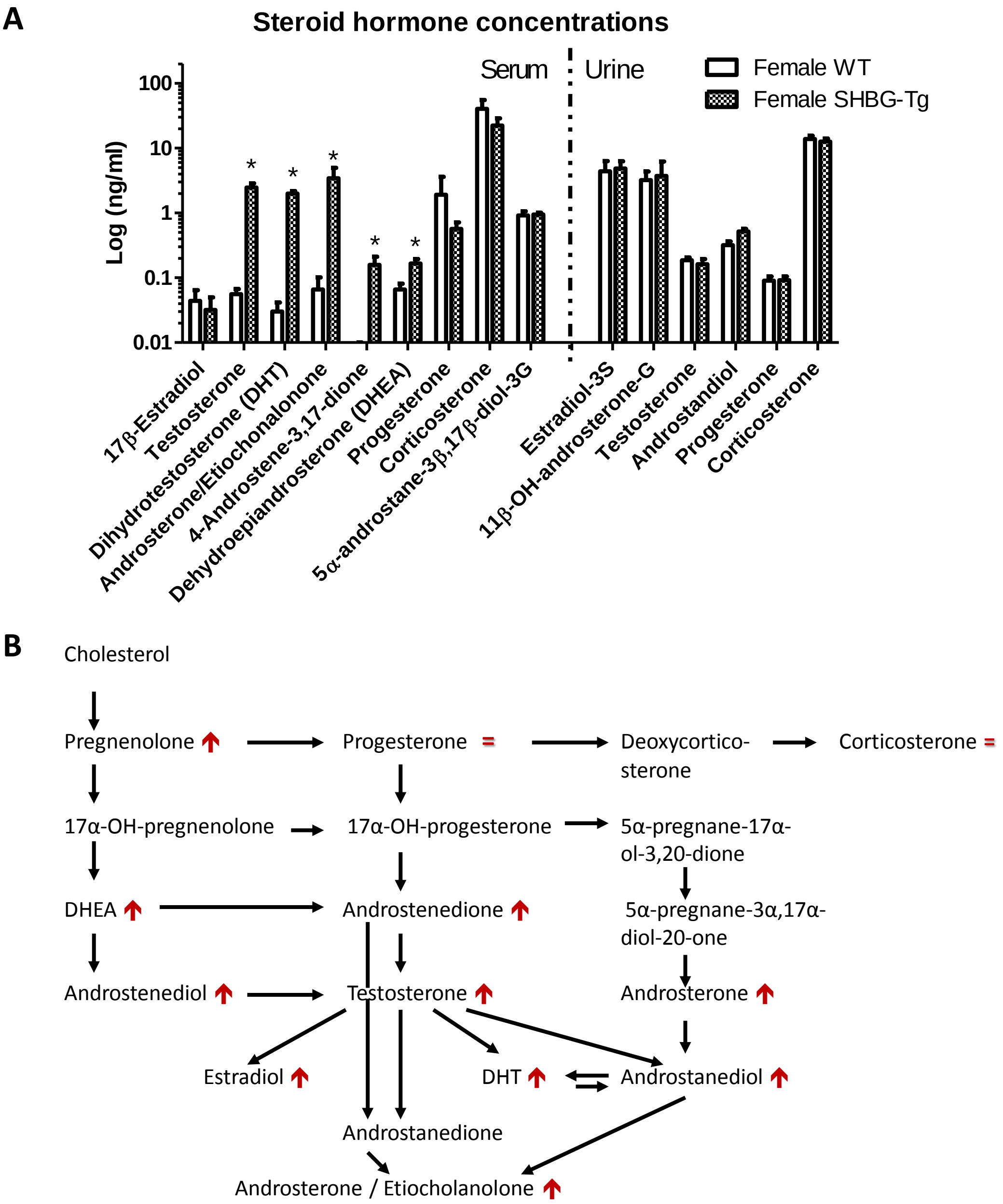

Fig. S1. Steroid hormone profile in SHBG-Tg mice.

A. Concentrations in serum (left from vertical dotted line) and urine (right from vertical dotted line) of selected steroid hormones in randomly cycling 24-week-old WT and SHBG-Tg female mice (n=5 per group). * = P<0.05 vs. WT mice.

B. Simplified diagram showing the metabolism of selected steroid hormones (assessed by our steroid profile LC-MS/MS method) along with summary of differences (shown in red) in SHBG-Tg vs. WT mice.

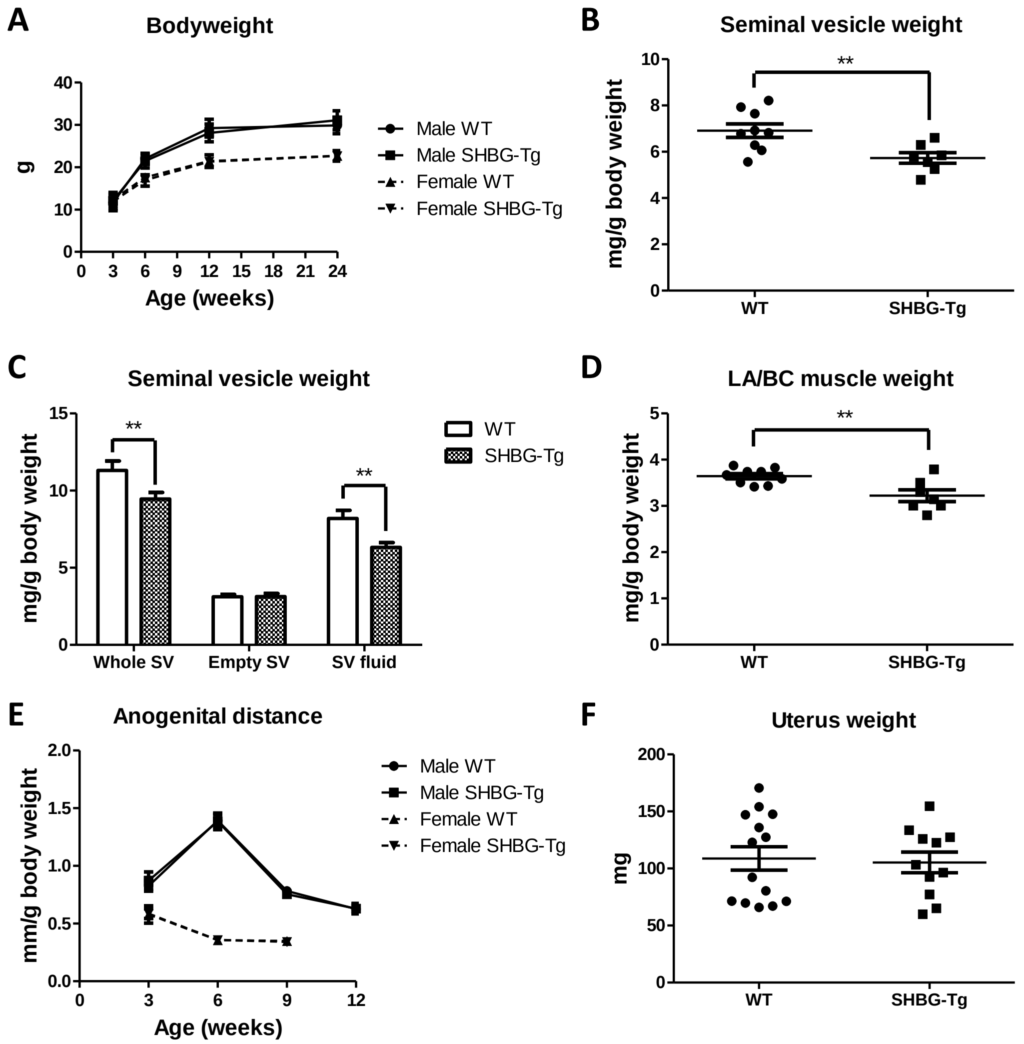

Fig. S2. Additional phenotyping of SHBG-Tg mice.

A. Body mass evolution in both genders and genotypes at different ages. n=8-17 per group

B. Seminal vesicle (SV) weights at 9 weeks of age. n=7-10 per group. ** = P<0.01

C. Weights of intact SVs, fluid-emptied organs and SV fluid. n=4-6 per group. ** = P<0.01 by Bonferroni's post-test after two-way ANOVA showing significant effect of fluid expression (P<0.0001), genotype (P=0.03) and interaction (P=0.001).

D. Levator ani/bulbocavernosus complex (LA/BC) muscle weights at 9 weeks of age. n=7-10 per group. ** = P<0.01

E. Anogenital distance evolution in both genders and genotypes at different ages across puberty. Note consistently higher levels in males, and decrease in males from age 6 weeks due to faster body weight gains than anogenital separation. Mean ± SD shown, n=5-13 per group and timepoint.

F. Uterus weights of WT and SHBG-Tg mice at 24 weeks of age. n=11-14 per group.

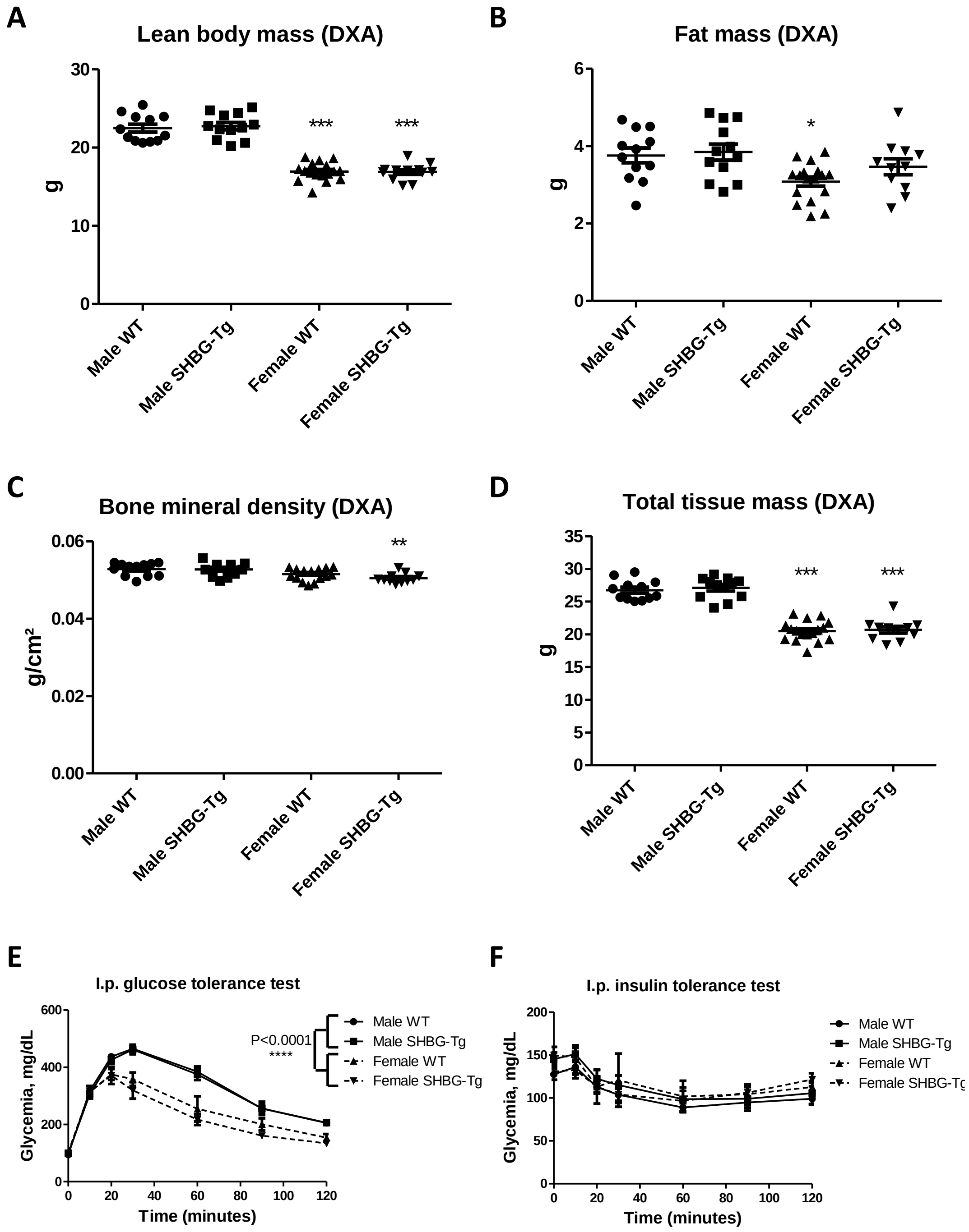

Fig. S3. Normal body composition and glucose homeostasis in SHBG-Tg mice.

A-D. Lean body mass, fat mass, bone mineral density and total tissue mass by *in vivo* dual-energy X-ray absorptiometry (DXA) in 24-week-old male and female WT and SHBG-Tg mice. n=11-17 per group. * = P<0.05, ** = P<0.01, *** = P<0.001 compared to WT males.

E, F. Glycemic responses to an i.p. glucose or insulin tolerance test. n=7-8 (males) and n=4-6 (females) for each genotype.

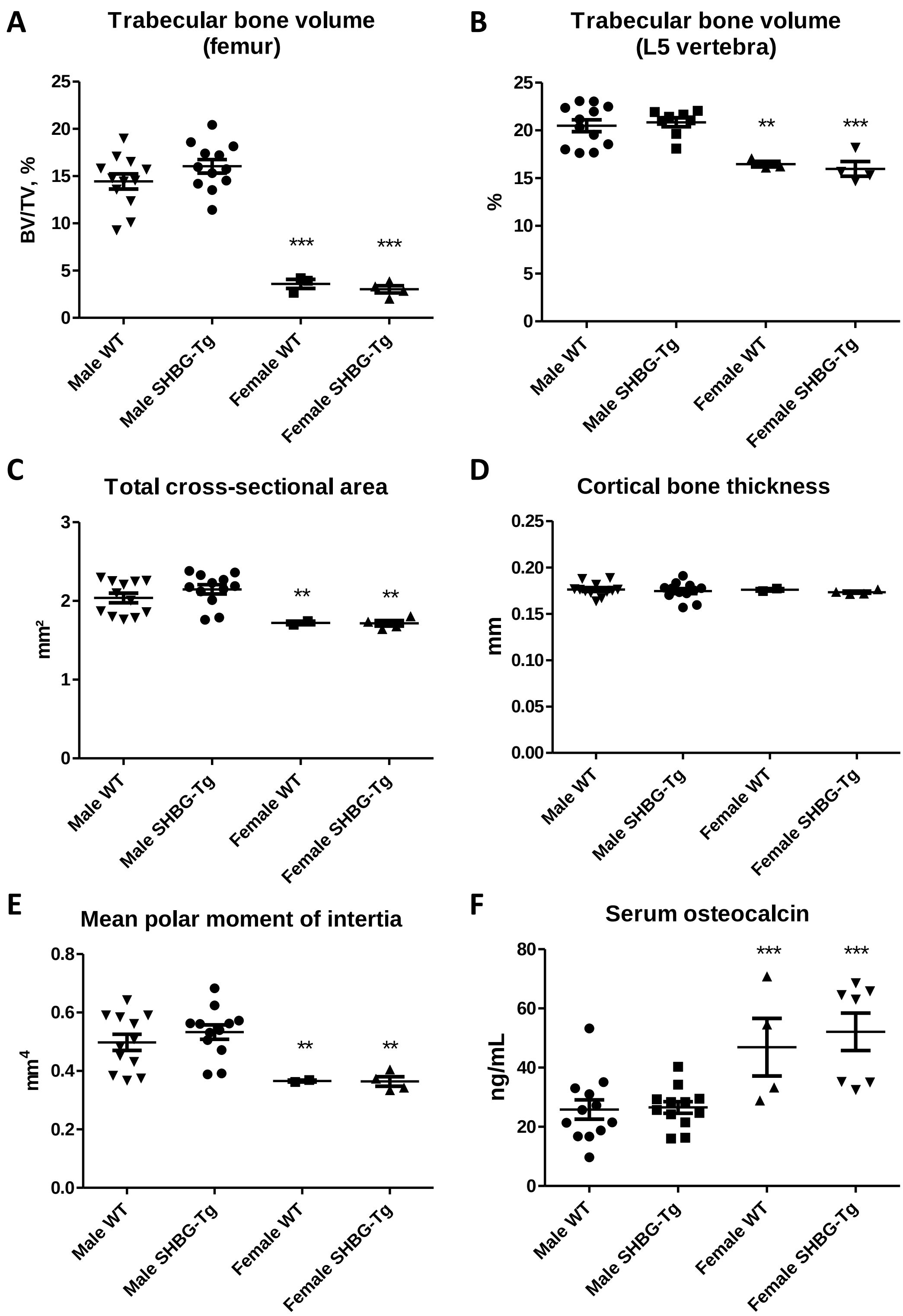

Fig. S4. Lack of bone phenotype in 24-week-old SHBG-Tg mice.

A. Trabecular bone volume (BV/TV) in the distal femur. n=12 (males) and n=3-4 (females) for each genotype. *** = P<0.001 compared to WT males.

B. Trabecular bone volume (BV/TV) in the L5 vertebra. n=8-12 (males) and n=3-4 (females) for each genotype. ** = P<0.01, *** = P<0.001 compared to WT males.

C. Total cross-sectional bone area (an indicator of periosteal circumference) of the femoral midshaft. n=12 (males) and n=3-4 (females) for each genotype. ** = $P<0.01$ compared to WT males.

D. Cortical bone thickness of the femoral midshaft. n=12 (males) and n=3-4 (females) for each genotype.

E. Mean polar moment of intertia of the femoral midshaft. n=12 (males) and n=3-4 (females) for each genotype. ** = $P<0.01$ compared to WT males.

F. Serum osteocalcin. n=12 (males) and n=4-7 (females) for each genotype.