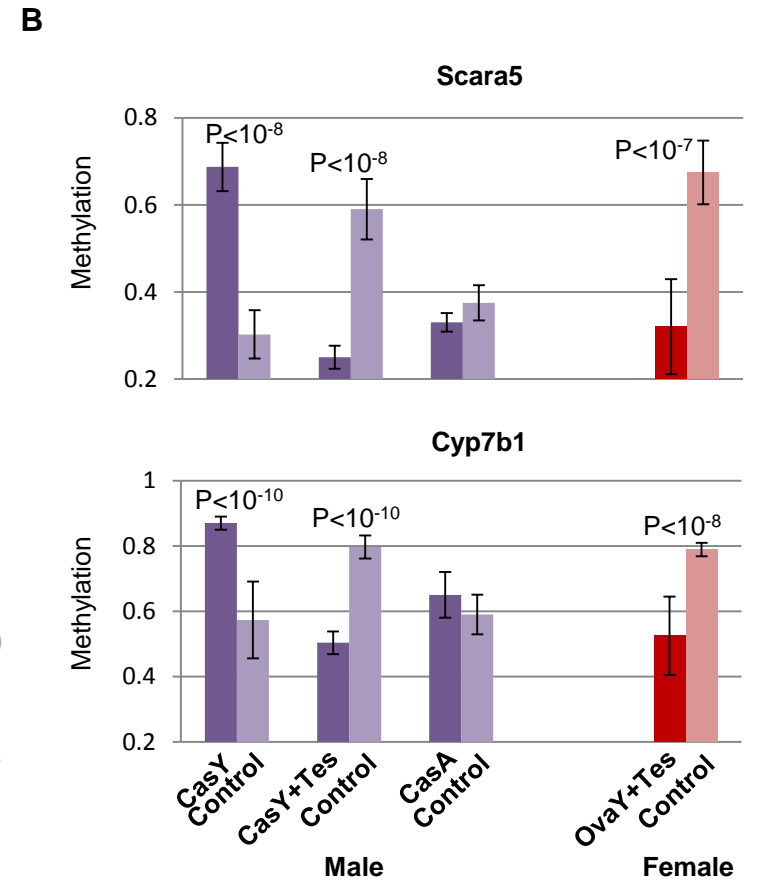
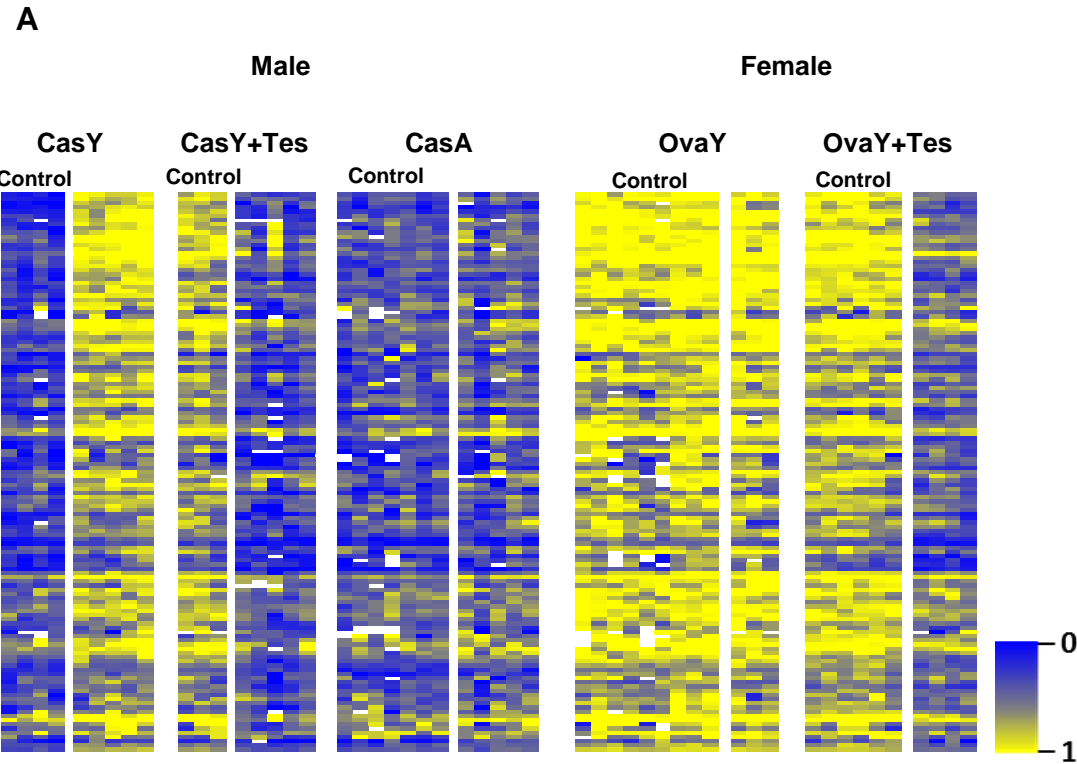


**Figure S1: Demethylation in male liver.** (A) Heatmap (RRBS) of the 160 male-female liver-differentially-methylated tiles (Figure 1) in different tissues from males and females and in E6.5–E7.5 embryos. (B) Whole genome bisulfite heatmap and average methylation levels (C) of individual liver samples from 20 d and 20 wk males and a 20 wk female for the same 160 differentially-methylated tiles detected by RRBS (Figure 1). (D) WGBS heatmap and average methylation levels (E) of 1630 400 bp tiles differentially methylated (> 35%) between male and female liver. (F) Average methylation levels for the 160 male-female differentially methylated tiles measured in purified hepatocytes from males (M) and females (F).

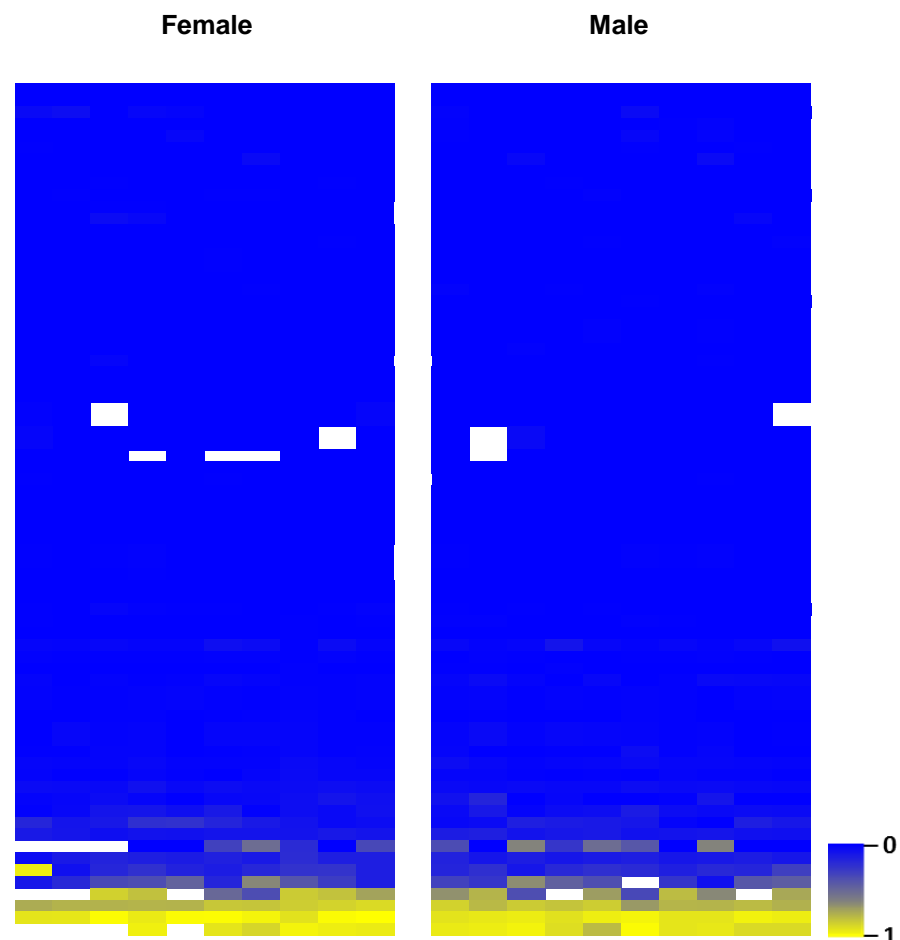


**Figure S2 : Effect of testosterone.** (A) Full heatmap of liver DNA methylation levels of the 160 differential tiles (Figure 1) for all WT, control, castrated and testosterone treated animals shown in Figure 2A. (B) Average methylation levels ( $\pm$ SD) of two specific tiles located within the genes Scara5 and Cyp7b1 were validated by independent Bisulfite deep sequencing for all of the mice shown in Figure 2.

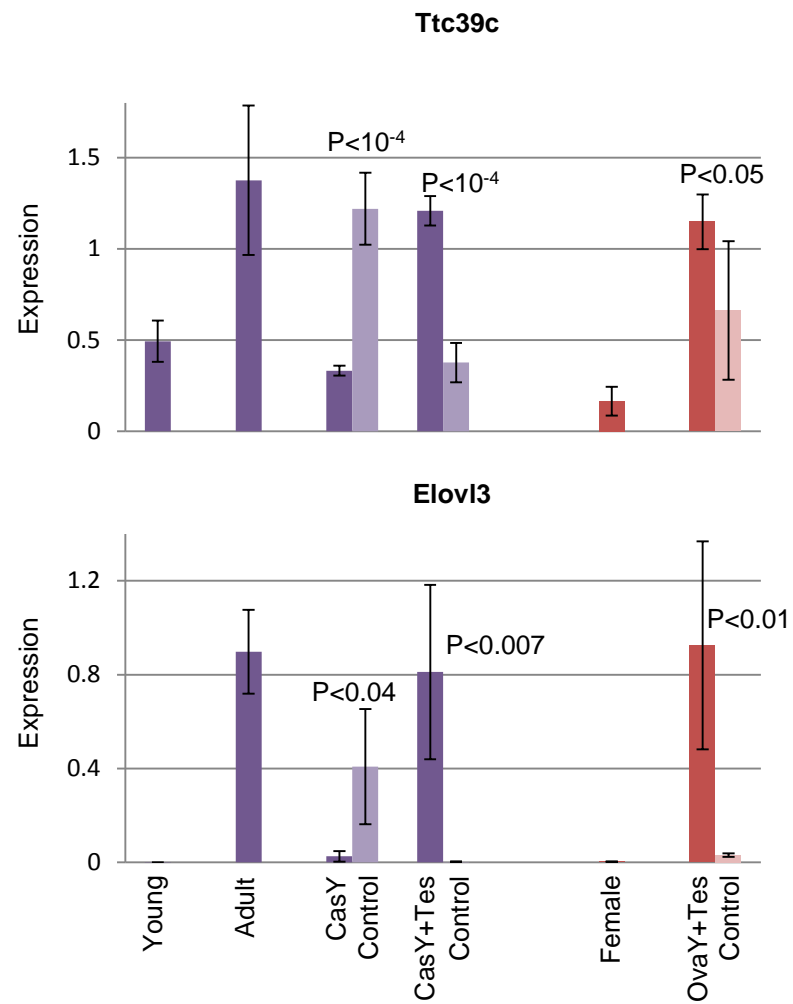
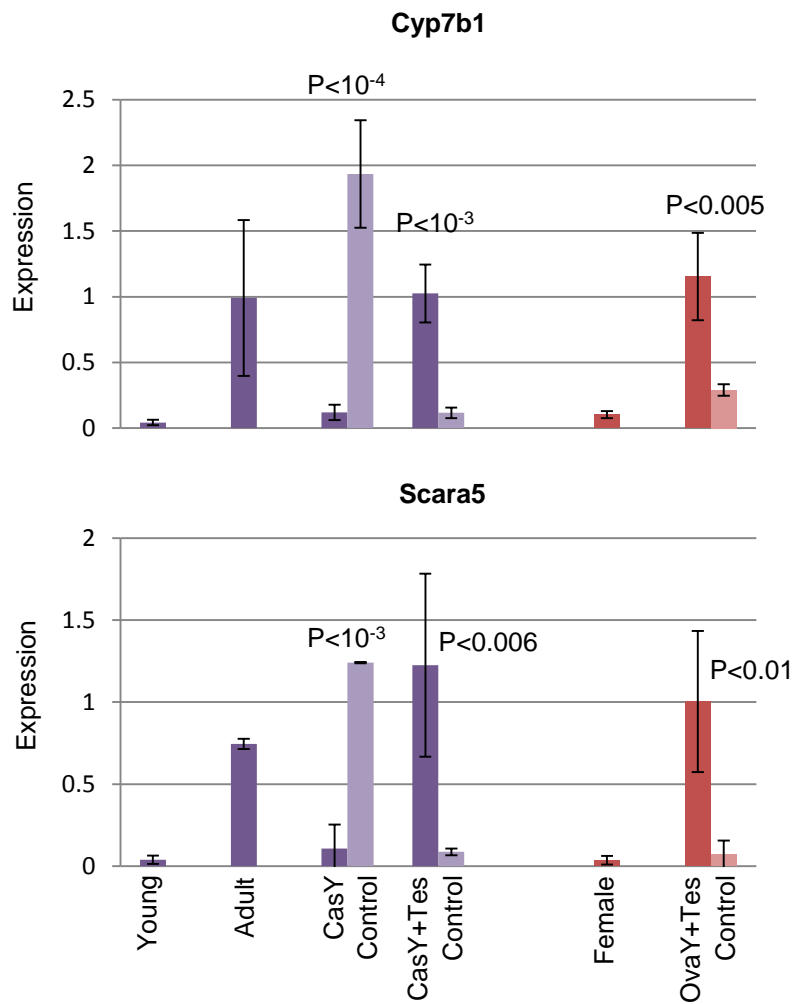
A

TFBS Name	Promoter Association	Z-score
HNF6	-	8.9
HNF1	-	6.23
STAT	-	5.91
BCL6	-	4.34
ZF02	+	-11.51
EGRF	+	-11.93
E2FF	+	-12.62
ZF5F	+	-14.05

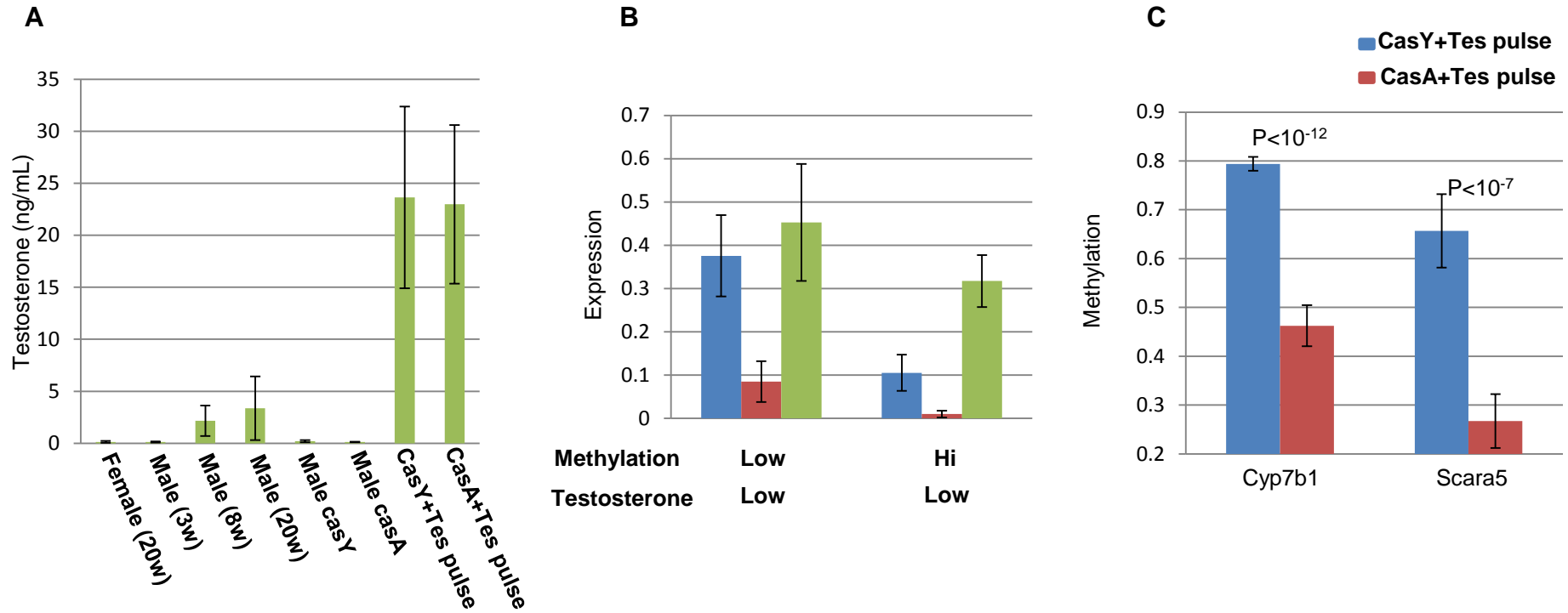
B



**Figure S3: Promoter characterization.** (A) Motif analysis (Genomatix) of differentially methylated regions (RRBS) showing enrichment for transcription factor binding sites (TFBS) that are not associated with promoters. 38 different TFBSs are over-represented ( $Z\text{-score} > 4$ ) and none of these are associated with promoters. In contrast, 70% of the TFBSs that are under-represented ( $Z\text{-score} < -4$ ) show strong association with promoters (see examples in table). (B) Heatmap of promoter methylation for genes (WGBS,  $n=72$ ) that correlate with enhancer demethylation as determined by RRBS using multiple individual samples of liver DNA from males and females. No significant difference in methylation was observed between male and female samples.



**Figure S4: Expression validation.** Expression levels (RT-PCR) ( $\pm$ SD) of four representative male-differential genes (Figure 4C), including controls. Male (blue), female (red).



**Figure S5.** (A) Blood testosterone levels ( $\pm$ SD) in normal and treated mice. Males were castrated at 3 wk (CasY) or at 19 wk (CasA), given testosterone at 20 wk and assayed 2 days later. (B) Expression levels (RT-PCR) ( $\pm$ SD) of three genes (Cyp7b1-blue, Scara5-red, Ttc39c-green) in male liver (28 wk) from animals castrated at 3 wk (methylated enhancers) or castrated at 20 wk (undermethylated enhancers) ( $n=10$ ,  $P<0.05$ , t-test). (C) Methylation levels ( $\pm$ SD,  $n>3$  biological repeats) of target sites in the Scara5 and Cyp7b1 gene regions after castration and a testosterone pulse (control for Figure 5B).