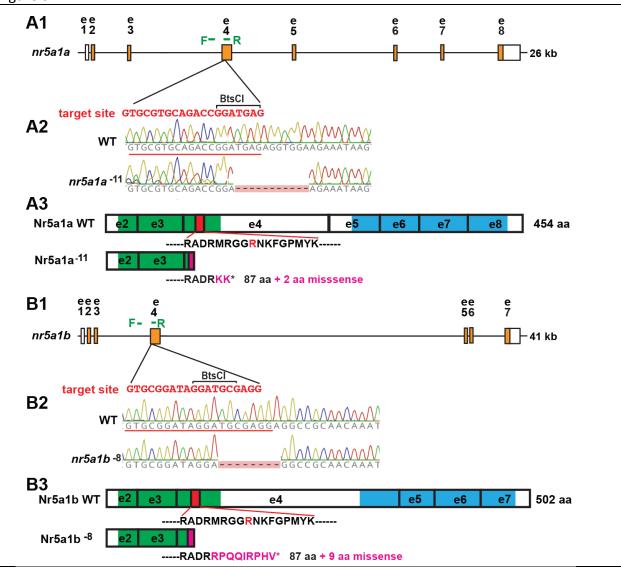
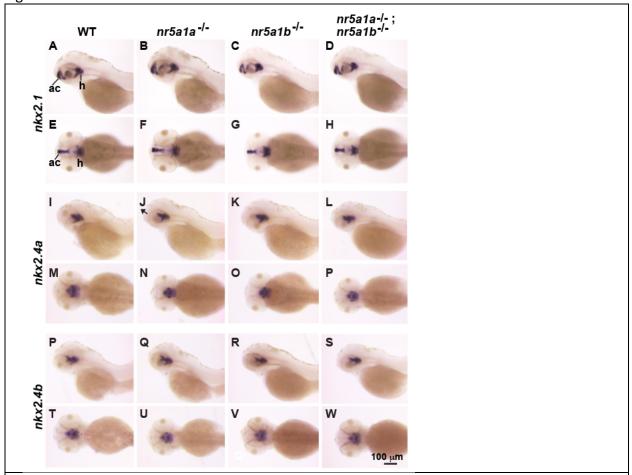
Figure S1



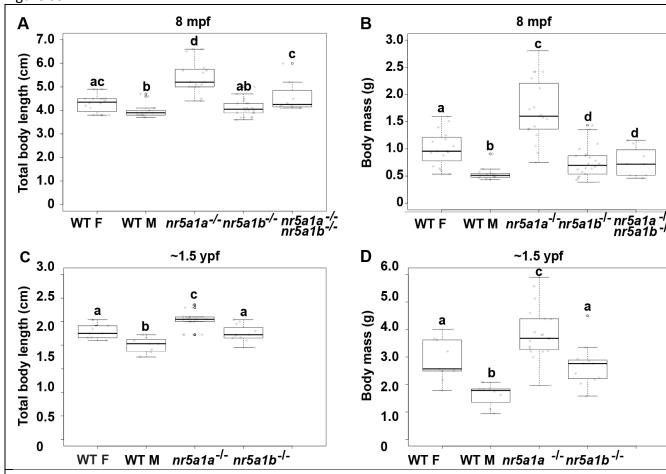
Supplemental Figure S1. Molecular genetics of *nr5a1a* and *nr5a1b* mutations. **A1**. A 26kb genomic section containing zebrafish nr5a1a showing the CRISPR target site in exon-4 (red letters). Forward (F) and reverse (R) PCR primers (green). The location of the BtsCl restriction enzyme site (black line) containing the polymorphism used for genotyping nr5a1a mutants. A2. Sequence traces from *nr5a1a* genomic DNA from a wild-type fish and from a -11bp deletion homozygous fish. A3. Predicted structure of the Nr5a1a wild-type protein and the predicted out-of-frame portion (pink); premature stop codon (*). The wild-type protein is 454 amino acids (aa) long; the mutant protein is predicted to have normal 87 amino acid residues and two missense residues resulting from the out-of-frame deletion. B1. A 41 kb genomic section containing the zebrafish nr5a1b gene showing the CRISPR target site sequence in exon-4 (red). Forward (F) and reverse (R) PCR primers (green). The location of the BtsCl restriction enzyme site (black line) containing the polymorphism used for genotyping nr5a1b mutants. B2. Seguence traces from nr5a1b genomic DNA from a wild-type fish and from an -8 bp deletion homozygous fish. B3. Predicted structure of the Nr5a1a wild-type protein and the predicted out-of-frame portion (pink); premature stop codon (*). The wild-type protein is 502 amino acids (aa) long; the mutant protein is predicted to have 87 appropriate amino acid residues and nine missense residues resulting from the out-of-frame deletion.

Figure S2



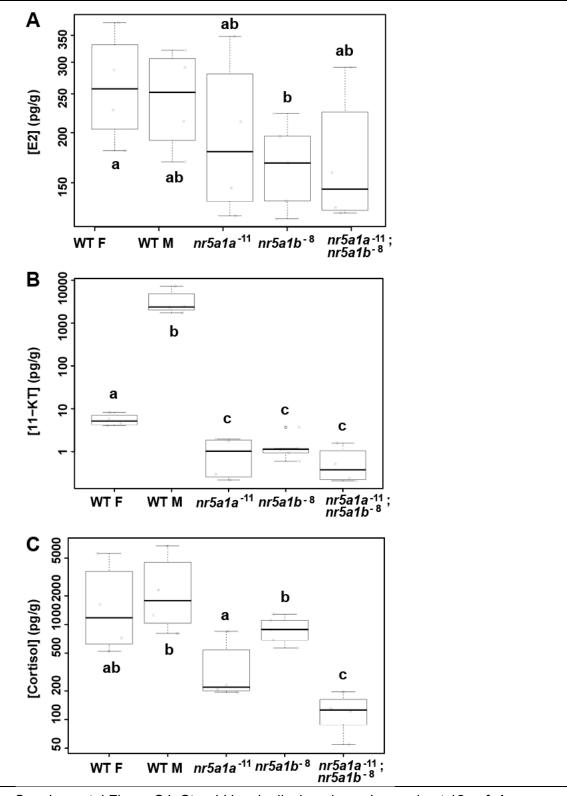
Supplemental Figure S2. Expression patterns of *nkx2.1*, *nkx2.4a* and *nkx2.4b* genes in 48hpf embryos. Wild types (A, E, I, M, P, T); *nr5a1a*⁻¹¹ mutants (B, F, J, N, Q, U); *nr5a1b*⁻⁸ mutants (C, G, K, O, R, V); *nr5a1a*⁻¹¹; *nr5a1b*⁻⁸ double mutants (D, H, L, P, S, W). In situ hybridization for *nkx2.1* (side view: A, B, C, D and dorsal view: E, F, G, H), *nkx2.4a* (side view: I, J, K, L and dorsal view: M, N, O, P), *nkx2.4b* (side view: P, Q, R, S and dorsal view: T, U, V, W). Neither *nr5a1* ohnolog appears to have a dramatic effect on the expression of any of the *nkx2*-family genes in the hypothalamus. Based on three clutches, each containing 20-25 wild types, 42-48 heterozygotes, 19-24 *nr5a1a* mutants, 18-27 *nr5a1b* mutants, and 8-12 double mutant embryos. Abbreviations: ac, anterior commissure; h, hypothalamus. Scale bar in W represents 100µm.

Figure S3



Supplemental Figure S3. Body length and weight in nr5a1 mutants. A. Total body length of 8mpf adults. B. Body weight of 8mpf adults. C. Total body length of adults at about 1.5 years post fertilization (ypf). D. Body weight of adults at about 1.5 ypf. Morphometrics of adult zebrafish. (A) Body length and (B) weight of 8-month post-fertilization zebrafish (WT F (wild-type females), n=16. WT M (wild-type males), n = 13. nr5a1a mutants, n= 19. nr5a1b mutants, n = 22. nr5a1a;nr5a1b double mutants, n = 8.). Body length (C) and body weight (D) of zebrafish about 1.5 years old (WT F (wild-type females), n=3. WT M (wild-type males), n = 2. nr5a1a mutants, n = 10. nr5a1b mutants, n = 7).

Figure S4



Supplemental Figure S4. Steroid levels displayed as a log scale at 12mpf. A. Estradiol (E2) levels (pg/g) in $nr5a1a^{-11}$, $nr51b^{-8}$ single mutants, $nr5a1a^{-11}$; $nr5a1b^{-8}$ double mutants and wild-type siblings. B. 11-ketotestosterone (11 KT) levels (pg/g) in $nr5a1a^{-11}$, $nr51b^{-8}$ single mutants, $nr5a1a^{-11}$; $nr5a1b^{-8}$ double mutants and wild-type siblings. C. Cortisol levels (pg/g) in $nr5a1a^{-11}$, $nr51b^{-8}$ single mutants, $nr5a1a^{-11}$;

 $nr5a1b^{-8}$ double mutants and wild-type siblings. WT F (wild-type females), n=4. WT M (wild-type males), n = 4. nr5a1a mutants, n = 4. nr5a1b mutants, n = 5. nr5a1a;nr5a1b double mutants, n = 4. Statistical significances indicated by different letters (a-e, p<0.05).