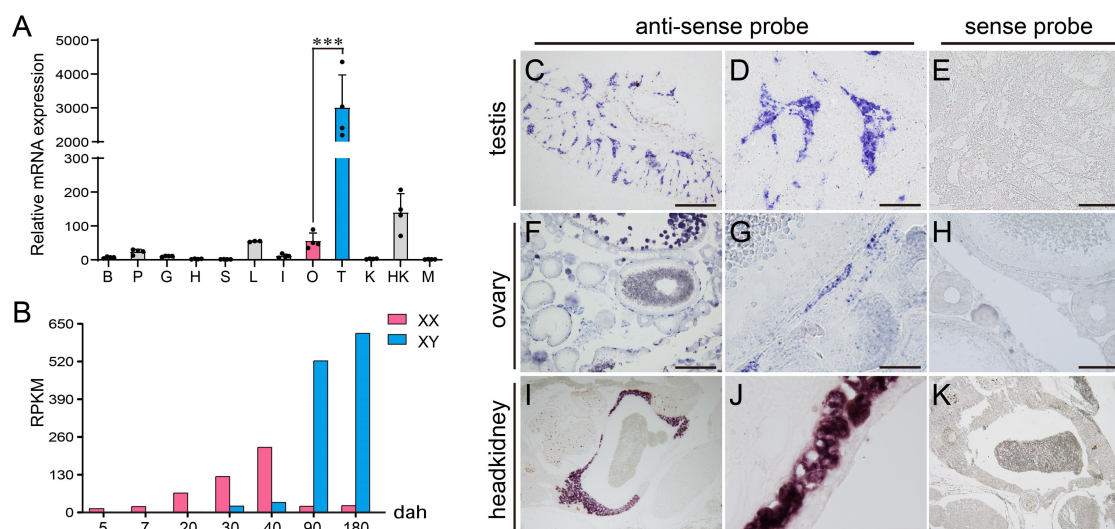
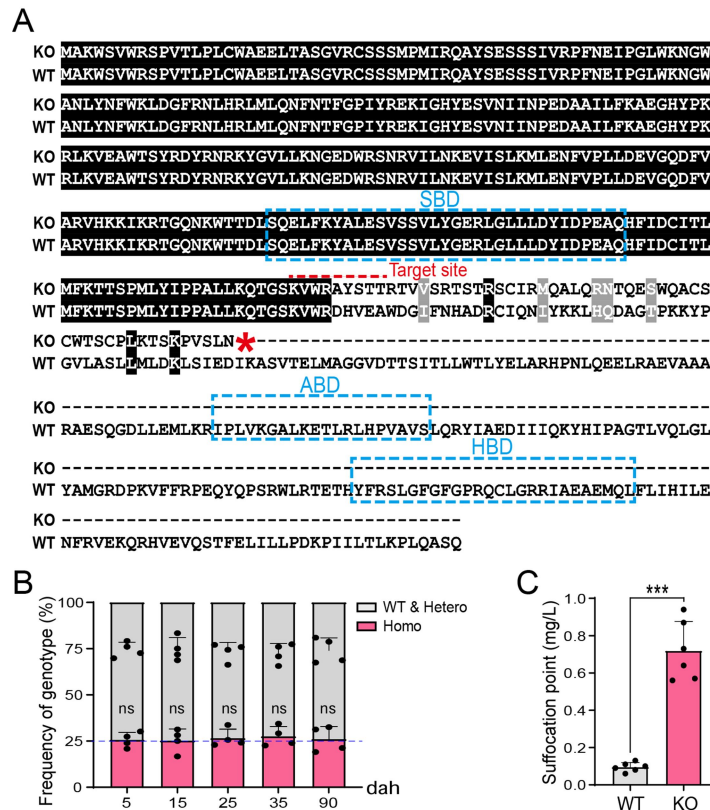


Supplementary information



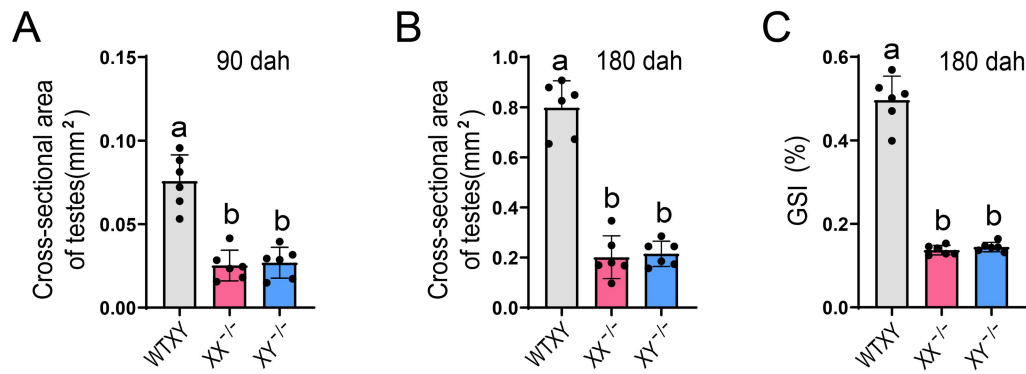
Supplementary Figure 1: Spatial and temporal expression of tilapia *cyp11a1*

A) Relative mRNA expression level of *cyp11a1* in twelve tissues of tilapia. Values are presented as mean $\pm$ SD (n=4). Difference in ovary and testis was determined by two-tailed independent Student's *t*-test. “****” above the error bar indicates statistically significant differences at $P < 0.001$. **B)** The expression of *cyp11a1* in the gonads of tilapia at different developmental stages by transcriptome analysis. The reads per kb per million mapped reads (RPKM) method was used to calculate the gene expression level. **C-K)** Cellular location of *cyp11a1* in testis (C-E), ovary (F-H) and head kidney (I-K) of tilapia by ISH. Blue and brown color represents positive signal in C-H and I-K, respectively. Scale bar in C, F, I, K, 100 μ m; Scale bar in D, E, G, H, 25 μ m; Scale bar in J, 10 μ m. dah, days after hatching.



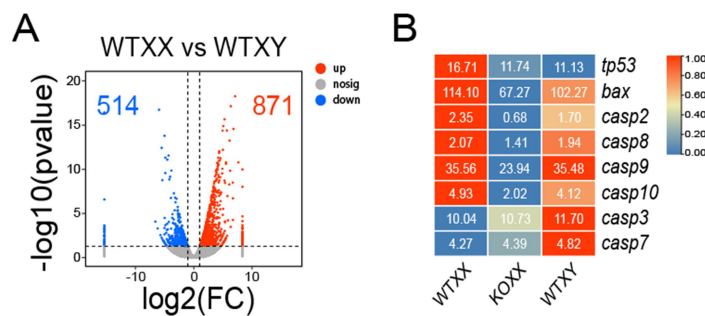
Supplementary Figure 2: *cyp11a1* mutation led to truncated protein and decreased anti-hypoxic ability

A) *cyp11a1* mutation led to a truncated Cyp11a1 protein lacking adrenodoxin binding domain (ABD) and heme binding domain (HBD) due to premature termination. SBD, substrate binding domain. The target site is marked with red dashed line. Red asterisk indicates premature termination. **B)** Frequency of wild type fish (WT), heterozygous (Hetero) and homozygous mutants (Homo) in the F2 population at 5, 15, 25, 35 and 90 dah (n=4 populations at each time point, more than 48 fish were randomly selected in each population). The blue dashed line indicates the theoretical proportion of homozygous mutants in the F2 population according to Mendel's Law. **C)** Suffocation point of the WT and *cyp11a1*^{-/-} fish at 90 dah (n=6/genotype). Values are presented as mean ± SD. The frequency of genotype in B at each time point were determined by Chi-squared test. The difference in C was determined by two-tailed independent Student's *t*-test. "****" above the error bar indicates statistically significant differences at *P*<0.001. ns, not significant. dah, days after hatching.



Supplementary Figure 3: Cross-sectional area of testes and gonadosomatic index of WT XY, *cyp11a1*^{-/-} XX and *cyp11a1*^{-/-} XY fish.

A-B) Cross-sectional area of testes of WT XY, *cyp11a1*^{-/-} XX and *cyp11a1*^{-/-} XY fish at 90 (A) and 180 (B) dah. **C)** GSI of WT XY, *cyp11a1*^{-/-} XX and *cyp11a1*^{-/-} XY fish at 180 dah. Values are presented as mean ± SD. Different letters above the error bar indicate statistically significant differences at P<0.05 as determined by one-way ANOVA followed by Turkey test for multiple comparisons. GSI, Gonadosomatic index. dah, days after hatching.



Supplementary Figure 4: Gonadal transcriptome analysis of WT XX, WT XY, *cyp11a1*^{-/-} XX fish at 15 dah

A) Volcano plot of DEGs in the gonads of WT XX and WT XY fish. **B)** The expression of genes related to apoptosis in the gonads of the WT XX, WT XY, *cyp11a1*^{-/-} XX fish. The number inside the square is the RPKM value of genes in each sample. The color from blue to red represents the expression of genes from low to high in each sample.