## **Description of Additional Supplementary Files:**

Supplementary Data 1: annotated SRY locus - CLC main workbench file - SRY - Complete locus

**Supplementary Data 2:** annotated SRY locus - CLC main workbench file - SRY - 7386bp sequence fragment

**Supplementary Data 3:** annotated SRY locus - CLC main workbench file - SRY - 83 species sequence list

**Supplementary Data 4:** annotated SRY locus - CLC main workbench file - SRY - 83 species sequence alignment

**Supplementary Data 5:** annotated SRY locus - CLC main workbench file - SRY - 17 species sequence alignment

Supplementary Data 6: List of the mammalian species used in this study

Supplementary Data 7: Genomatix predicted transcription binding sites in the E250 enhancer

Supplementary Data 8: H1a1 haplogroup

**Supplementary Data 9:** Luciferase: annotated Plasmid-pcDNA3-Myc2X-NR5A1 - CLC main workbench file

**Supplementary Data 10:** Luciferase: annotated Plasmid-pCMV-Renilla-AF025843 - CLC main workbench file

**Supplementary Data 11:** Luciferase: annotated Plasmid-pGL4.26\_link5\_E250-Reference - CLC main workbench file

**Supplementary Data 12:** Luciferase: annotated Plasmid-pGL4.26\_link5\_pGL4.26\_link5\_E250-Variant-1

**Supplementary Data 13:** Luciferase: annotated Plasmid-pGL4.26\_link5\_pGL4.26\_link5\_E250-Variant-2

**Supplementary Data 14:** Luciferase: annotated Plasmid-pGL4.26\_link5\_E250-ΔNR5A1 - CLC main workbench file

Supplementary Data 15: Luciferase on HEK293T cells - statistical analysis in R

Supplementary Data 16: Luciferase on HeLa cells - statistical analysis in R

**Supplementary Data 17:** RT-qPCR analysis - AMH - Wild type versus mutant comparison

Supplementary Data 18: RT-qPCR analysis - SRY - Linear Mixed Model in R

**Supplementary Data 19:** RT-qPCR analysis - SOX9 - Linear Mixed Model in R

Supplementary Data 20: RT-qPCR analysis - WNT4 - Linear Mixed Model in R

Supplementary Data 21: RT-qPCR analysis - NR5A1 - Linear Mixed Model in R

**Supplementary Data 22:** RT-qPCR analysis - FOXL2 - Linear Mixed Model in R

**Supplementary Data 23:** RT-qPCR analysis - FGF9 - Linear Mixed Model in R

Supplementary Data 24: RT-qPCR analysis - SRY - data preparation for Linear Mixed Model in R

Supplementary Data 25: RT-qPCR analysis - SOX9 - data preparation for Linear Mixed Model in R

Supplementary Data 26: RT-qPCR analysis - WNT4 - data preparation for Linear Mixed Model in R

Supplementary Data 27: RT-qPCR analysis - NR5A1 - data preparation for Linear Mixed Model in R Supplementary Data 28: RT-qPCR analysis - FOXL2 - data preparation for Linear Mixed Model in R Supplementary Data 29: RT-qPCR analysis - FGF9 - data preparation for Linear Mixed Model in R Supplementary Data 30: RT-qPCR analysis - SRY - outlier identification by the IQR method in R Supplementary Data 31: RT-qPCR analysis - SOX9 - outlier identification by the IQR method in R Supplementary Data 32: RT-qPCR analysis - WNT4 - outlier identification by the IQR method in R Supplementary Data 33: RT-qPCR analysis - NR5A1 - outlier identification by the IQR method in R Supplementary Data 34: RT-qPCR analysis - FOXL2 - outlier identification by the IQR method in R Supplementary Data 35: RT-qPCR analysis - FGF9 - outlier identification by the IQR method in R **Supplementary Data 36:** RT-qPCR analysis - SRY - Wild type versus mutant comparison **Supplementary Data 37:** RT-qPCR analysis - SOX9 - Wild type versus mutant comparison Supplementary Data 38: RT-qPCR analysis - WNT4 - Wild type versus mutant comparison Supplementary Data 39: RT-qPCR analysis - NR5A1 - Wild type versus mutant comparison **Supplementary Data 40:** RT-qPCR analysis - FOXL2 - Wild type versus mutant comparison **Supplementary Data 41:** RT-qPCR analysis - FGF9 - Wild type versus mutant comparison

**Supplementary Data 42:** RT-qPCR analysis - iPS07-Wild type expression kinetics

**Supplementary Data 43:** RT-qPCR analysis - iPS09-Wild type expression kinetics

**Supplementary Data 44:** RT-qPCR analysis - iPS12-Wild type expression kinetics

**Supplementary Data 45:** RT-qPCR analysis - iPS26a-Wild type expression kinetics

**Supplementary Data 46:** RT-qPCR analysis - iPS26b-Wild type expression kinetics

**Supplementary Data 47:** RT-qPCR analysis: GraphPad Prism file summarizing all RT-qPCR experiment results

Supplementary Data 48: Statistical tests - Figure 4 and Figure 5