**Supplementary table 1. Sybr green primer pairs for RT-PCR**

|  |  |  |
| --- | --- | --- |
| Gene | Forward/Reverse Primer | Primer |
| *Gapdh* | *Forward Primer* | *AGGTCGGTGTGAACGGATTTG* |
|  | *Reverse Primer* | *TGTAGACCATGTAGTTGAGGTCA* |
| *Acta2* | *Forward Primer* | *GTCCCAGACATCAGGGAGTAA* |
|  | *Reverse Primer* | *TCGGATACTTCAGCGTCAGGA* |
| *Cyp1b1* | *Forward Primer* | *CACCAGCCTTAGTGCAGACAG* |
|  | *Reverse Primer* | *GAGGACCACGGTTTCCGTTG* |
| *Cldn1* | *Forward Primer* | *GGGGACAACATCGTGACCG* |
|  | *Reverse Primer* | *AGGAGTCGAAGACTTTGCACT* |
| *P2rx2* | *Forward Primer* | *GCGTTCTGGGACTACGAGAC* |
|  | *Reverse Primer* | *ACGTACCACACGAAGTAAAGC* |
| *Fabp7* | *Forward Primer* | *GGACACAATGCACATTCAAGAAC* |
|  | *Reverse Primer* | *CCGAACCACAGACTTACAGTTT* |
| *Slitrk1* | *Forward Primer* | *GAAGGGGACTTACACGTAGACT* |
|  | *Reverse Primer* | *AGTGAGGGAATTGCCATGCAG* |
| *Ajap1* | *Forward Primer* | *ACAGAGTTCAACGATTTCGACTT* |
|  | *Reverse Primer* | *GGGAAGGTGTTGGACTCCAG* |
| *Itga9* | *Forward Primer* | *AAGTGTCGTGTCCATACCAAC* |
|  | *Reverse Primer* | *GGTCTGCTTCGTAGTAGATGTTC* |
| *Hsd11b2* | *Forward Primer* | *GGTTGTGACACTGGTTTTGGC* |
|  | *Reverse Primer* | *AGAACACGGCTGATGTCCTCT* |
| *Gfra2* | *Forward Primer* | *CGCCTCTCGGACATCTTCAG* |
|  | *Reverse Primer* | *TTGCAGTTGTCGTTCAGGTTG* |
| *Syn3* | *Forward Primer* | *CAATGGCTACATGCCGGAC* |
|  | *Reverse Primer* | *CCTGGGTCTTTGAACCACGG* |
| *Trpc6* | *Forward Primer* | *AGCCAGGACTATTTGCTGATGG* |
|  | *Reverse Primer* | *AACCTTCTTCCCTTCTCACGA* |
| *Astn2* | *Forward Primer* | *GGTGGACTCATTGCACTTCTC* |
|  | *Reverse Primer* | *TTGCGCTCTTCTGTGGGATG* |
| *Xkr4* | *Forward Primer* | *ACTTCGCGGATGTGGGAAC* |
|  | *Reverse Primer* | *CCAGCGGAAGCTGAACACTT* |
| *Fgf5* | *Forward Primer* | *TGTGTCTCAGGGGATTGTAGG* |
|  | *Reverse Primer* | *AGCTGTTTTCTTGGAATCTCTCC* |
| *Tgfbi* | *Forward Primer* | *CAGCACGGCCCCAATGTAT* |
|  | *Reverse Primer* | *GGGACCTTTTCATATCCAGGACA* |
| *Defa-rs4* | *Forward Primer* | *AACGGCCTGCTTACCTTACT* |
|  | *Reverse Primer* | *ACGTGTTCAGGTTCAACCCC* |
| *Dpt* | *Forward Primer* | *TGGATGGGTGAATCTTAACCGC* |
|  | *Reverse Primer* | *TCAGAGCCTTCCTTCTTGCTA* |
| *Sytl5* | *Forward Primer* | *GGAAGACAAGAGGATAAGGAAGC* |
|  | *Reverse Primer* | *TTGTGACAGTGAACGCAGACT* |
| *Zcchc5* | *Forward Primer* | *TGCCCCTAGAGTACCCTTTGG* |
|  | *Reverse Primer* | *TCAGTGGGATACCCTCTGCTT* |
| *Pdlim3* | *Forward Primer* | *TGGGGGCATAGACTTCAATCA* |
|  | *Reverse Primer* | *CTCCGTACCAAAGCCATCAATAG* |
| *slc38a5* | *Forward Primer* | *CTACAGGCAGGAACGCGAAG* |
|  | *Reverse Primer* | *GGTTGAACACTGACATTCCGA* |
| *Enpp2* | *Forward Primer* | *TTTGCACTATGCCAACAATCGG* |
|  | *Reverse Primer* | *GGAGGCACTTTAGTCCTGTACTT* |
| *Dubr-202* | *Forward Primer* | *ATAGCAACGGCCTGCTTACC* |
|  | *Reverse Primer* | *GGTTCAACCCCTCGGTCTTT* |
| *Ampd3* | *Forward Primer* | *GTTGGCGGAGAAGGTGTTTG* |
|  | *Reverse Primer* | *CTGCGACCGGATCATCTTGAA* |
| *unc13c* | *Forward Primer* | *AAAGCGAACTGCAAAGTGATGA* |
|  | *Reverse Primer* | *TGCTCTCTGAAGAGTAGATCCAA* |
| *Aspn* | *Forward Primer* | *AAGGAGTATGTGATGCTACTGCT* |
|  | *Reverse Primer* | *ACATTGGCACCCAAATGGACA* |
| *Col6a6* | *Forward Primer* | *GTGGACAGCTCCGATCACCTA* |
|  | *Reverse Primer* | *GGGCGTTCCCTATCTTCAGG* |
| *Col6a4* | *Forward Primer* | *CTTCTGACAGCCGTACTGCC* |
|  | *Reverse Primer* | *CGAGCACGAATAATGCGATGC* |
| *Nrxn3* | *Forward Primer* | *TGGGCTGCCTTAAAGAGGTTG* |
|  | *Reverse Primer* | *GGGGTCTCAAAGTTGATGGGA* |
| *Gpr50* | *Forward Primer* | *AGAGCAACATGGGACCTACAA* |
|  | *Reverse Primer* | *GCCAGAATTTCGGAGCTTCTTG* |
| *Col14a1* | *Forward Primer* | *TTTGGCGGCTGCTTGTTTC* |
|  | *Reverse Primer* | *CGCTTTTGTTGCAGTGTTCTG* |
| *Pgm5* | *Forward Primer* | *TGCACCAGATGTTGTCTCAGA* |
|  | *Reverse Primer* | *CTTCCCAGTCGAGATAGGTCA* |
| *Nrk* | *Forward Primer* | *GACCTGGGAGTTGGAGGGA* |
|  | *Reverse Primer* | *CATAAGTACCAAGACCAATGGCT* |
| *Ano1* | *Forward Primer* | *CCCGTGCCAGTCACCTTTTT* |
|  | *Reverse Primer* | *TCATCTGCTTCCGTTTCCAGT* |
| *Col23a1* | *Forward Primer* | *CCCCATCTGAGTGCATCTGTC* |
|  | *Reverse Primer* | *CTTGCCGTCCAGACCTAGAG* |
| *Mfap4* | *Forward Primer* | *GCAACCCCTGGACTGTGATG* |
|  | *Reverse Primer* | *TTGTCATGTCGCAGAAGACGG* |
| *Enc1* | *Forward Primer* | *CTGTTTCATAAGTCCTCCTACGC* |
|  | *Reverse Primer* | *CACCACTGAACATGGCTTCG* |

**Supplementary table 2. The top 20 downregulated genes in the absence of *Coup-TFII* in the mesonephros.**

|  |  |  |
| --- | --- | --- |
| **Gene Symbol** | **p-value (KO vs. WT)** | **Fold change (KO vs WT)** |
| *Nr2f2* | 1.32E-06 | -2.51884 |
| *Cyp1b1* | 0.000187305 | -2.24913 |
| *Cldn1* | 0.000964991 | -1.87018 |
| *Amhr2* | 0.00027735 | -1.84531 |
| *P2rx2* | 0.000170114 | -1.76531 |
| *Fabp7* | 0.00126727 | -1.76238 |
| *Slitrk1* | 0.000666716 | -1.74521 |
| *Ajap1* | 3.20E-05 | -1.69407 |
| *Itga9* | 0.00109656 | -1.65097 |
| *Zpld1* | 0.00234644 | -1.58798 |
| *Tm4sf5* | 0.0026883 | -1.57505 |
| *Hsd11b2* | 0.00746169 | -1.55323 |
| *Gfra2* | 0.00339666 | -1.54512 |
| *Syn3* | 0.000105253 | -1.53617 |
| *Trpc6* | 0.000126465 | -1.51388 |
| *Astn2* | 0.000237335 | -1.49525 |
| *Xkr4* | 0.00489398 | -1.49496 |
| *Fgf5* | 0.00143297 | -1.49425 |
| *Tgfbi* | 0.000120038 | -1.49423 |
| *Defa-rs4* | 0.00750525 | -1.48824 |

**Supplementary table 3. The top 20 upregulated genes in the absence of *Coup-TFII* in the mesonephros.**

|  |  |  |
| --- | --- | --- |
| **Gene Symbol** | **p-value(KO vs. WT)** | **Fold change KO vs WT** |
| *Dpt* | 0.00279629 | 3.53294 |
| *Sytl5* | 0.00336484 | 2.20115 |
| *Ret* | 0.00086905 | 2.13712 |
| *Zcchc5* | 0.000359838 | 2.13014 |
| *Pdlim3* | 1.46E-05 | 2.10404 |
| *Slc38a5* | 1.89E-06 | 2.07221 |
| *Enpp2* | 0.00408385 | 2.0472 |
| *Dubr* | 0.0045779 | 2.04561 |
| *Ampd3* | 0.000446763 | 2.01873 |
| *Unc13c* | 7.46E-05 | 1.95979 |
| *Aspn* | 0.0473918 | 1.91231 |
| *Col6a6* | 0.00629587 | 1.87608 |
| *Col6a4* | 0.0305903 | 1.83411 |
| *Ano4* | 0.000156451 | 1.79544 |
| *Acta2* | 0.0013504 | 1.77138 |
| *Nrxn3* | 0.00786912 | 1.77108 |
| *Gpr50* | 0.0143848 | 1.76813 |
| *Col14a1* | 0.00350225 | 1.76605 |
| *Pgm5* | 0.000823237 | 1.76501 |
| *Nrk* | 0.00384636 | 1.73988 |

**Supplementary Table 4: Target molecules of top upstream regulators in the list of differentially expressed genes.**

|  |  |
| --- | --- |
| **Upstream regulators** | **Notes** |
| TGFB1 | ACTA2,ADAM12,ADM,ADORA2B,AGTR2,AIM2,ANGPT1,ANPEP,ANXA2,AQP1,ASPN,ATXN1,BMP2,BMP6,C1R,CADM1,CCL11,CD40,Cdkn1c,CELF2,CLDN1,Clec2d (includes others),COL6A3,CXCL13,CXCR6,DCN,DES,EGFR,ELN,ENPP1,ETS1,F3,FAS,FERMT1,FGF5,FGF7,FGFR2,FN1,FXYD6,GDF6,GEM,GMPR,GNA14,GPR12,GRM3,GRM7,GUCY2F,HEXB,HOXA2,HTRA1,ID4,IGFBP5,IGFBP7,INHBB,ITGA11,KITLG,KLK3,MAPK13,MFAP4,MGP,MYH11,MYOCD,P2RY1,PLAGL1,PLCB1,PLCL1,PPFIBP2,PTGER4,RHOB,RUNX1,S1PR3,SCD,SERPINF1,SLC16A9,SLC7A5,SLIT3,SPP1,SVEP1,TAGLN,TBX2,TGFBI,TGM2,THBD,THBS1,THBS2,THBS4,THY1,TLL1,TNFAIP6,TRIM9,TRPC1,VAT1L,VCAM1,VDR,WNT11,WT1 |
| progesterone | ACTA2,ADAMTS1,ADM,AEBP1,ALPL,AMPD3,AQP1,AR,BMP2,CA12,CADM1,CCNG1,CD40,Cdkn1c,CNR1,CYP1B1,EGFR,ENC1,ENPP1,ENPP2,F3,FGFR2,FN1,GREB1,HSD11B2,IGFBP5,IRS2,ITGA11,JAM2,KCNMA1,KLK3,LEPR,LGALS1,LGR5,LMO7,MME,NPY1R,PARM1,PLCL1,PRSS35,PTGER4,SCD,SPP1,ST3GAL1,TBX2,TGFBI,THBD,TNXB,TRPC1,VDR |
| beta-estradiol | ACTA2,ADAMTS1,ADAMTS18,ADAMTS5,ADM,AEBP1,AGTR2,ALX4,ANGPT1,ANPEP,ANXA1,AQP1,AR,BMP2,BMP5,CA12,CACNA1D,CCNG1,CD40,CELF2,CHRM3,CLDN1,CLDN11,Clec2d (includes others),CNR1,COL6A6,COLEC12,CXCL13,CYP1B1,DCLK1,DCN,DUSP10,DUSP6,EGFR,ENC1,ENPP2,ETS1,F3,FAS,FGF7,FGFR2,FHL2,FN1,FRK,GEM,GPR12,GREB1,HSD11B2,HTRA1,HTRA3,ID4,IGFBP5,IGFBP7,IL6ST,INHBB,IRS2,ITGA11,JAM2,KCNN3,KITLG,KLK3,LEPR,LGR5,LMO7,MACC1,MEDAG,MGP,MME,MPPED2,NPY1R,NRIP1,NRP2,NTN1,NTRK3,PLAC1,PRKCB,PRSS35,PTGER4,PTGFR,RET,RND3,RUNX1,RYR2,SFRP2,SLC7A5,SLC8A1,SLIT2,SMOC1,SNED1,SRD5A2,SSTR4,ST8SIA1,SYNE1,THBD,THBS1,THBS4,TNFAIP6,TP53INP1,TRIB2,TRPC1,VCAM1,VDR,WNT11 |
| TNF | ABCD2,ACTA2,ADAMTS5,ADM,ADORA2B,AEBP1,AKR1B10,AMPD3,ANGPT1,ANPEP,ANXA1,APCS,AQP1,AR,BMP2,BMP6,BMPER,CA12,CCL11,CD40,CDO1,CHSY3,CLDN11,CRISPLD2,CXCL13,CYP1B1,CYP27A1,DCN,DUSP10,DUSP6,EGFR,EMCN,ENPP2,ENPP3,ETS1,EXOC3L4,F3,FAS,FERMT1,FGF5,FGFR2,FN1,FRZB,GABRA5,GEM,GFRA2,GRM3,GSDMD,HEXB,IGFBP5,IL1RL2,IRS2,KITLG,KLK3,LAMA4,LGR5,MFSD2A,MGP,NRIP1,NTN1,OSMR,P2RX2,PDE7B,PDZD2,PTGFR,RGS5,RHOB,RND3,S1PR3,SCD,SCUBE1,SERPINB9,SERPINF1,SLC16A2,SLC8A1,SP7,SPP1,TAGLN,TGM2,THBD,THBS1,THBS2,THY1,TNFAIP6,TP53INP1,TRPC1,TRPC6,USP2,VCAM1,VDR |
| TWIST1 | ACTA2,ADAMTS1,AR,CCDC3,CD40,DCN,EGFL6,ENPP3,ETS1,FAS,FAT4,FGFR2,FIBIN,FN1,FREM2,IGFBP5,MFGE8,MME,OLFML3,PDZD2,SPP1,ST8SIA2,TGFBI,THBS2 |

**Supplementary Table 5: Moues phenotype of putative COUP-TFII target genes with no expression information in the MGI database**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Expression in fetal mesonephros** | **Mouse phenotype in reproduction** | **Notes** |
| *Ampd3* | Unknown | No |  |
| *Atrnl1* | Unknown | No |  |
| *As3mt* | Unknown | No |  |
| *Camk2d* | Unknown | No |  |
| *Chsy3* | Unknown | Yes | Abnormal seminal vesicle; male and female infertility |
| *Epb41l2* | Unknown | Yes | Small testis and abnormal spermatogenesis |
| *Epha6* | Unknown | Yes | abnormal seminal vesicle and epididymis |
| *F3* | Unknown | No |  |
| *Grk5* | Unknown | No |  |
| *Il6st* | Unknown | Yes | Implantation failure |
| *Mme* | Unknown | No |  |
| *Palld* | Unknown | Unknown |  |
| *Rnu11* | Unknown | Unknown |  |
| *Sned1* | Unknown | Unknown |  |
| *Sulf1* | Unknown | No |  |
| *Syn3* | Unknown | No |  |
| *Tbc1d9* | Unknown | Unknown |  |
| *Them7* | Unknown | Unknown |  |
| *Trpc6* | Unknown | No |  |
| *Usp2* | Unknown | Yes | Abnormal testis and reduced male fertility. |
| *Xkr4* | Unknown | Unknown |  |

**Supplementary Table 6: Expression pattern and mouse phenotype of putative COUP-TFII target genes expressed in fetal mesonephros**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Expression in fetal mesonephros** | **Mouse phenotype in reproduction** | **Notes**  (There will be no notes if expression localization in the mesonephros cannot be defined) |
| *Abcd2* | Yes | No |  |
| *Ano1* | Yes | No | **Wolffian duct mesenchyme** and coelomic epithelium |
| *C1galt1* | Yes | No |  |
| *Cacna1c* | Yes | Unknown |  |
| *Cadm1* | Yes | No | Epithelium |
| *Cd24a* | Yes | Yes |  |
| *Col23a1* | Yes | Unknown | **Wolffian duct mesenchyme** |
| *Cyp27a1* | Yes | No |  |
| *Enc1* | Yes | No | **Wolffian duct mesenchyme** |
| *Fgfr2* | Yes | Yes |  |
| *Gem* | Yes | No |  |
| *Hmga2* | Yes | Yes |  |
| *Kcnj8* | Yes | No |  |
| *Lama1* | Yes | unknown | Expressed in Wolffian duct epithelium and mesenchyme |
| *Lama4* | Yes | No |  |
| *Lsp1* | Yes | No | Wolffian duct mesenchyme? |
| *Man1a* | Yes | Unknown | Wolffian duct epithelium? |
| *Mfap4* | Yes | No | **Wolffian duct mesenchyme** |
| *Mybpc1* | Yes | unknown | Epithelium of Wolffian and Müllerian ducts |
| *Nr2f2* | Yes | Yes | **Mesenchyme** |
| *Slc7a5* | Yes | Unknown | Epithelium? |
| *Nrip1* | Yes | Yes | Epithelium |
| *Nrk* | Yes | Yes |  |