

Figure S1: Somatic cell manipulation of $\beta$-catenin in the developing gonad E14.5 (A) Control ovary (No Cre;Ctnnb1F/F) and (B) mutant ovary (Sf1Cre;Ctnnb1F/F) $\beta$-catenin green, DAPI blue. The remaining $\beta$-catenin in the mutant ovary resides in the germ cell membrane. E14.5 (C) Control testis (No Cre;Ctnnb1 ${ }^{\Delta e x 3 /+}$ ) and (D) mutant testis (Sf1Cre;Ctnnb14ex3/+ $) ~ \beta$-catenin green, DAPI blue. $\beta$-catenin is highly stabilized in the mutant testis.



Figure S3: Chromatin Immunoprecipitation controls RNA Pol II is enriched at the GAPDH promoter and TCF7L2 is enriched at the SP5 promoter in both ovaries and testes


Figure S4: Validation of $\beta$-catenin specific responsiveness for CMV-S37A expression vector TOPflash and FOPflash constructs were cotransfected with $50 \mathrm{ng} / \mathrm{well}$ CMV-EGFP or CMVS37A and normalized to pGL3Basic. Only TOPflash co-transfected with CMV-S37A showed a specific and robust increase in luciferase expression.


Figure S5: Epigenetic marks on Irx3 and Irx5 promoters
H3K27me3 (red peaks and back solid lines) and open chromatin sites (ATAC-Seq, black peaks) are shown for $\operatorname{Irx} 3$ (C) and $\operatorname{Irx5}(\mathbf{D})$ promoters in male and female somatic cell populations. Arrows in label match the direction for coding sequences of each gene. ATAC-Seq peaks are enriched while there is a paucity of H3K27me3 peaks in XX samples. Black bars represent regions of significant enrichment when compared to flanking regions as determined by HOMER, thicker lines represent increased enrichment. Black bars are absent in both proximal promoter regions in XX samples.

| Site Label | Forward Primer |  | Reverse Primer | Total insert length |
| :---: | :---: | :---: | :---: | :---: |
| +205kb |  |  | $\qquad$ | 108bp |
| +86kb | $\stackrel{5}{5}-$GCGCGGTACCTTCCCTTTCCTATTTGTTCAGAAG-3 |  | $5^{5}-$ GCGCCTCGAGTTCCTCGGCTGAC AGAG | 59bp |
| -305AB kb | $\begin{aligned} & \text { G'- } \\ & \text { GCGCGGTACCGGTTTCAAAAAGCCCAA } \\ & \text { GTG-3' } \end{aligned}$ |  |  | 250bp |
| -580kb | $5^{5}-$GCGCGGTACCCCGCCATGATAGGAGTCAAC-3' |  | $5 ’-$ GCGCCTCGAGGGCAGCCCTTTGTA AATGTT-3' | 89bp |
| Mutation Site | +205kb | +86kb | -305kb (AB) | -580kb |
| Wild Type Sequence | GTTCAAAGGC | GTTCAAAGCG | (A) GTTCAAAGTC <br> (B) TTTCAAAGGG | CATCAAAGAC |
| Mutated Sequence | GTCCAAAGGC | GTCCAAAGCG | (A) GTCCAAAGTC <br> (B) TTCCAAAGGG | CACCAAAGAC |

Table S1: Individual potential enhancer sites containing TCF/LEF motif were cloned into the pGL3 Basic backbone using Kpnl and Xhol. Primer sequences listed above and the insert size. DNA was generated by PCR with mouse genomic DNA. Wild type and mutated TCF/LEF binding motif for each enhancer site. The mutated base pair is in bold.

Supplementary Table S2: Real-time qPCR primer sequences

| Gene | Forward Primer | Reverse Primer |
| :---: | :---: | :---: |
| 36B4 | 5' - CGACCTGGAAGTCCAACTAC - 3' | 5' - ATCTGCTGCATCTGCTTG - ${ }^{\prime}$ |
| Gapdh | 5' - TTCACCACCATGGAGAAGGC - 3' | 5' - GGCATGGACTGTGGTCATGA - 3' |
| Rps29 | 5' - TGAAGGCAAGATGGGTCAC - 3' | 3' - GCACATGTTCAGCCCGTATT - 5' |
| Axin2 | 5' - CCAGGCTGGAGAAACTGAAACT - 3' | 5' - CCTGCTCAGACCCCTCCTTT - 3' |
| Fst | 5' - AAAACCTACCGCAACGAATG - 3' | 5' - TTCAGAAGAGGAGGGCTCTG - 3' |
| Bmp2 | 5' - CGGACTGCGGTCTCCTAA - 3' | 5' - GGGGAAGCAGCAACACTAGA - 3' |
| Irx3 | 5' - CGCCTCAAGAAGGAGAACAAGA - 3' | 5' - CGCTCGCTCCCATAAGCAT - 3' |
| Irx5 | 5' - GGCTACAACTCGCACCTCCA - 3' | 5' - CCAAGGAACCTGCCATACCG - ${ }^{\prime}$ |

