

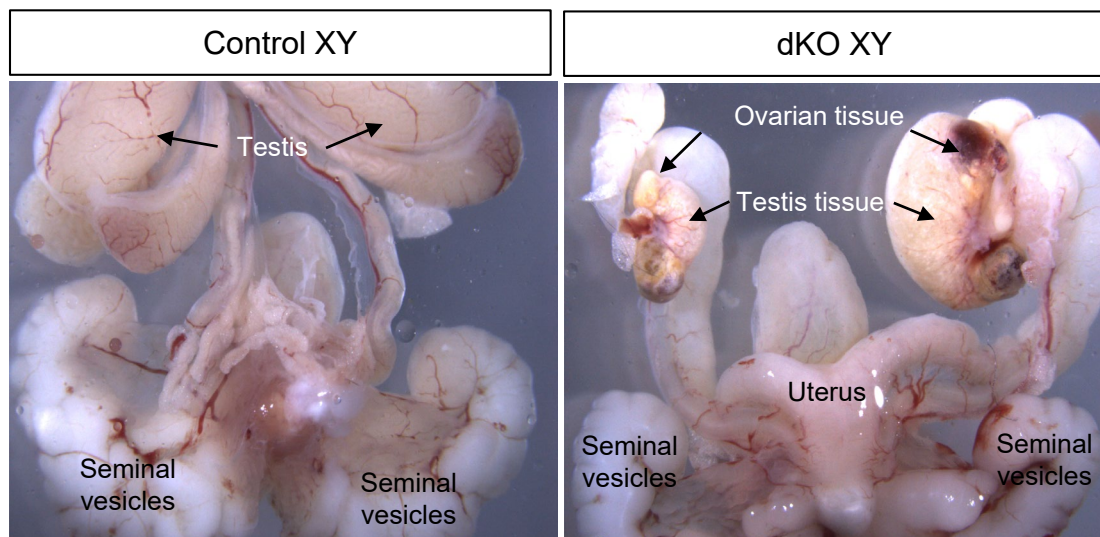
Supplemental Table 1

	Control XY	dKO XY	Control XX
Interstitial (XY)	7377	3438	4
Proliferating Interstitial (XY)	3724	3347	9
Fetal Leydig	1868	1423	0
Supporting (XY)	761	1205	0
Supporting (XY) 2	34	899	8
Supporting (XX)	0	93	4173
Stroma (XX)	520	989	4470
Epithelial	84	84	342
Germ	24	695	485
Endothelial	242	376	339
Immune	258	286	81
Pericytes	481	531	171
Erythrocytes	14	32	29
Total cells	15387	13852	10111

Supplemental Table 2

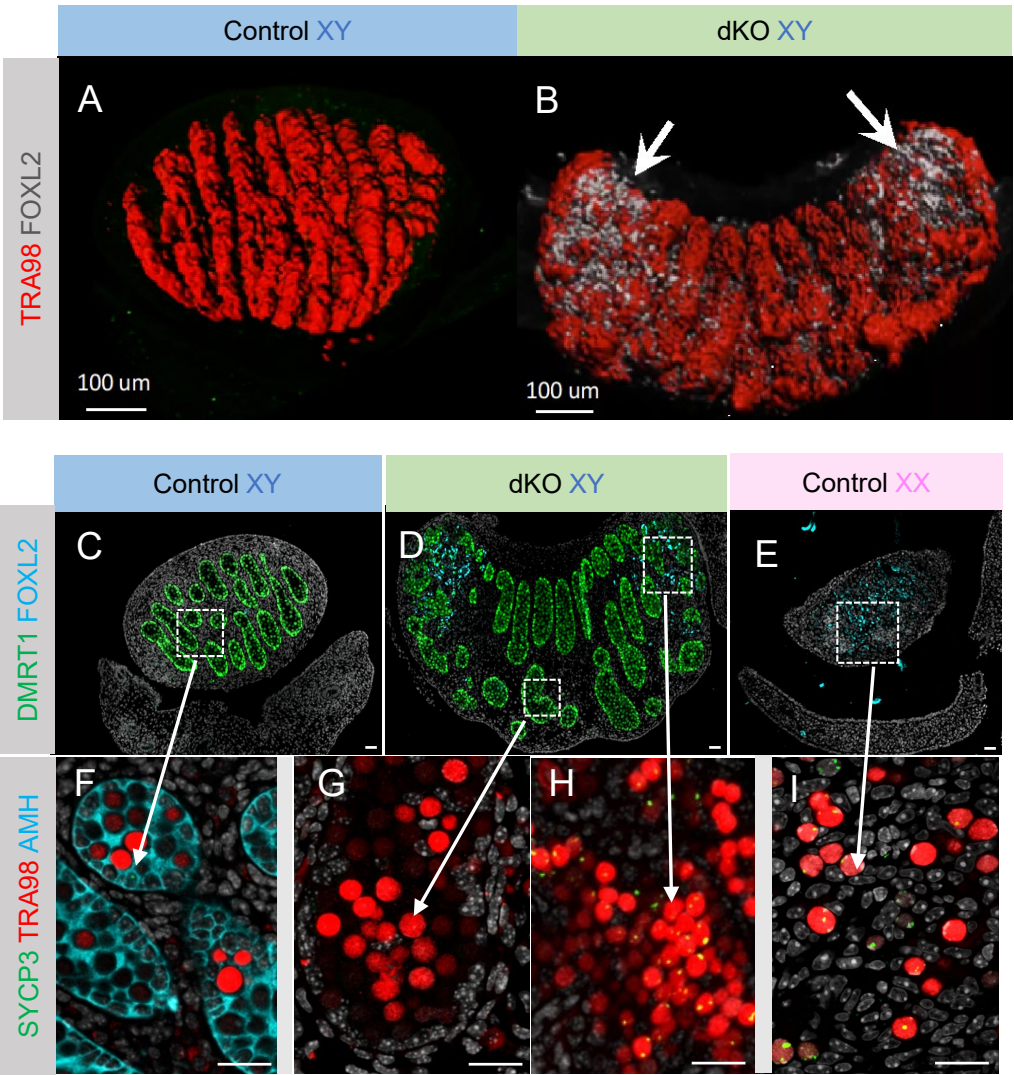
	Control XY	dKO XY	Control XX
XX Supporting 1	0	51	2254
XX Supporting 2	0	4	976
XX Supporting 3	2	19	403
dKO Feminized	33	827	15
XY Supporting 1	565	6	0
XY Supporting 2	33	1108	0
Total cells	633	2015	3648

Supplemental Figure 1



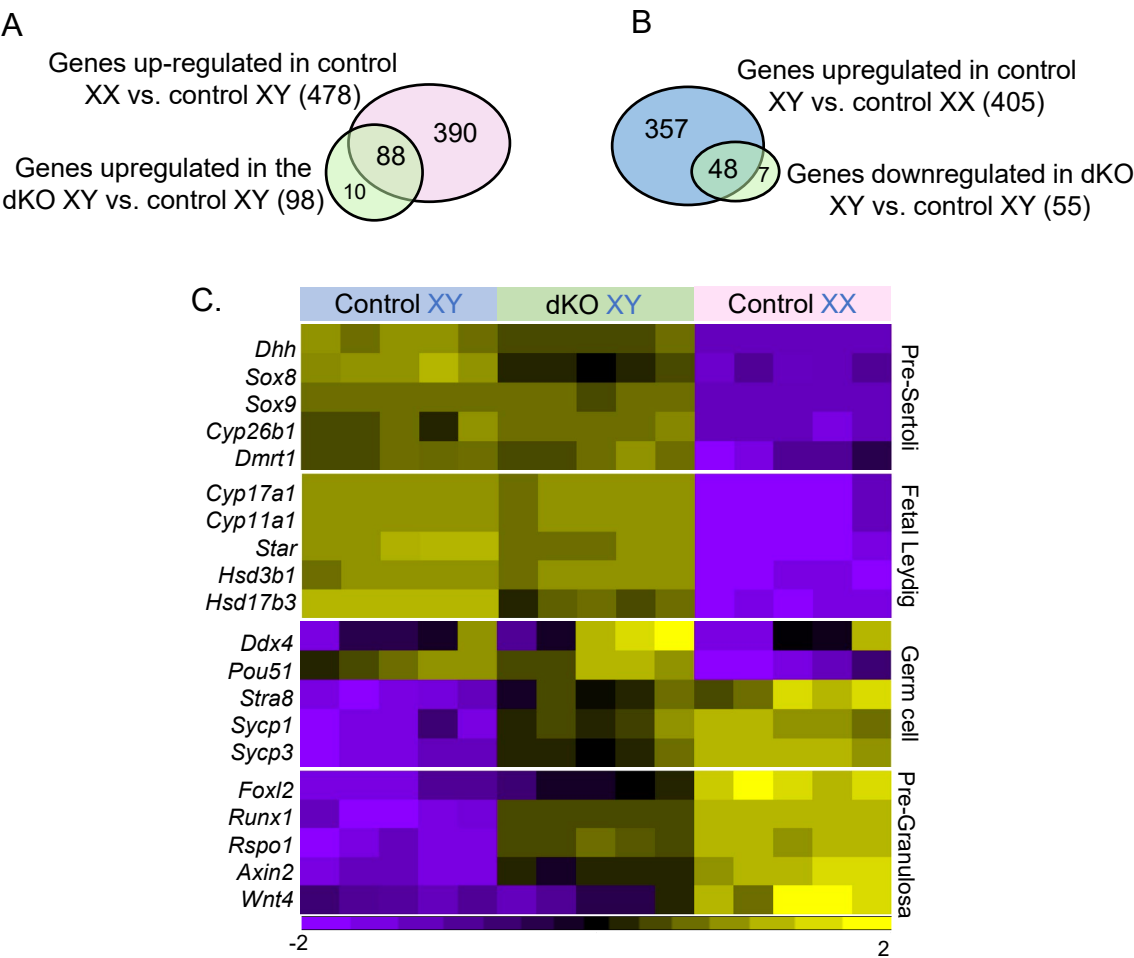
Supplemental Figure 1: Reproductive tracts from control XY and dKO XY adult mice. The dKO XY had the expected male reproductive organs such as seminal vesicles and epididymides; however, they also retained female reproductive organs including uterus. Ovarian tissues were visibly present along with the testis tissue. Variability on the size of the ovotestis was observed in adult dKO animals (n=12).

Supplemental Figure 2



Supplemental Figure 2: (A-B) Immunofluorescence for TRA98 and FOXL2 in control and dKO XY gonads at E15.5. Arrows indicate the ovarian structure in the poles. (C-I) Immunofluorescence for DMRT1, FOXL2, SYCP3, TRA98, and AMH in control XY, dKO XY, and control XX gonads at E15.5. Scale bar = 25 μ m.

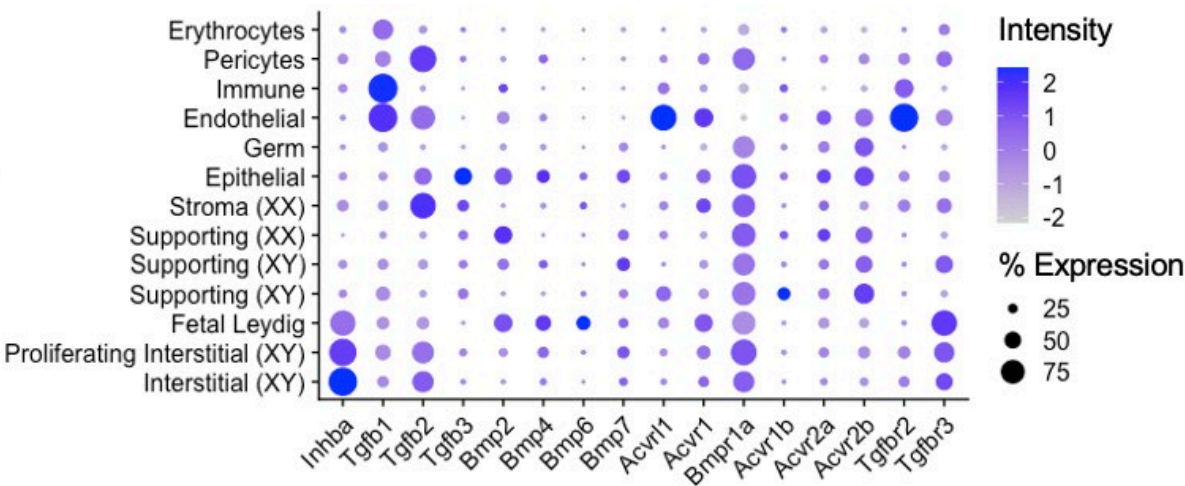
Supplemental Figure 3



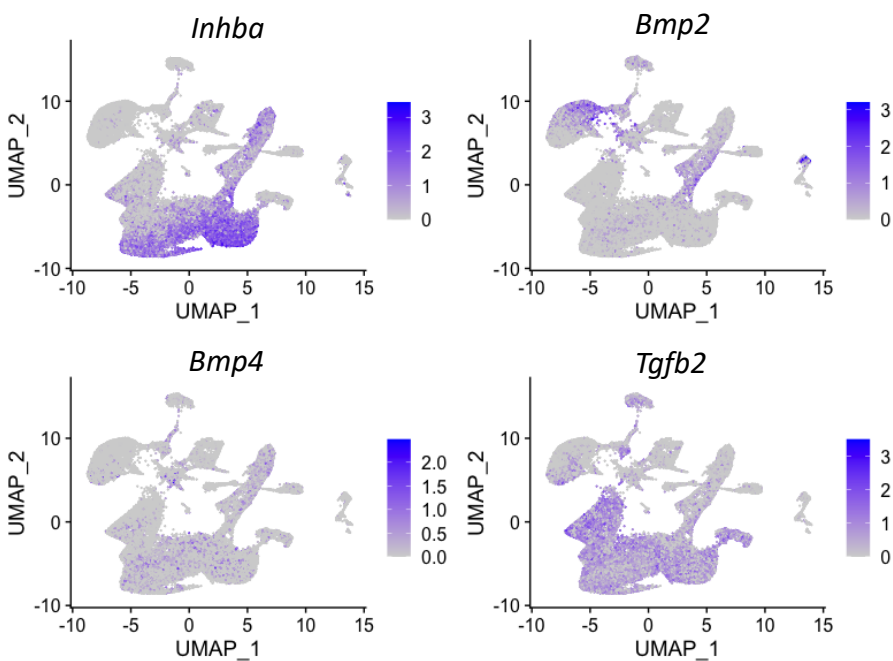
Supplemental Figure 3: (A-B) Transcriptome analysis of control XY, dKO XY, and control XX gonads at E15.5 (Source data are provided as a Source Data file; one-way ANOVA, $p < 0.05$ FDR). (C) Heat maps of genes critical for sex determination and gonadal development in control XY, dKO XY, and control XX gonads at E15.5. Each column represents the expression of a single gonad. Yellow and purple indicate up-regulation and down-regulation, respectively.

Supplemental Figure 4

A

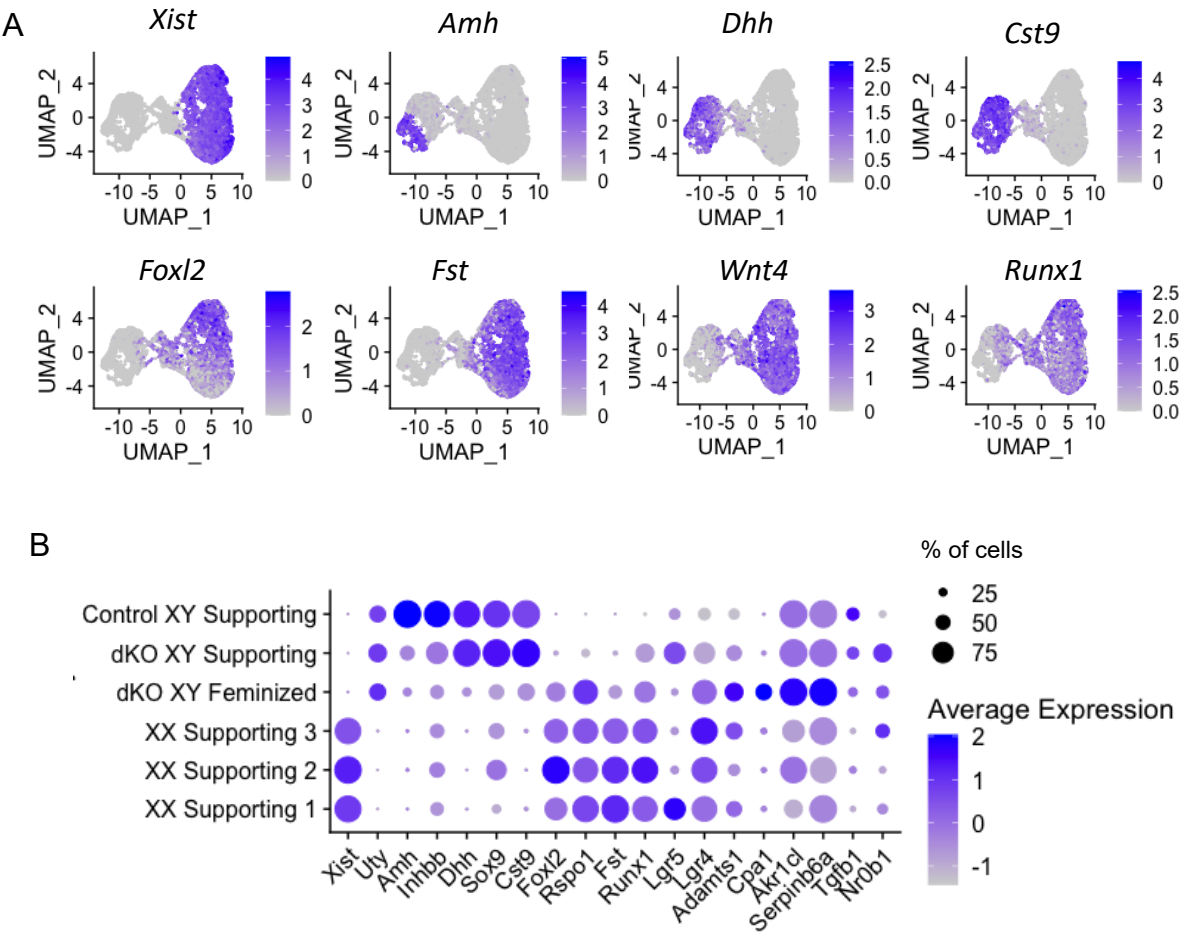


B



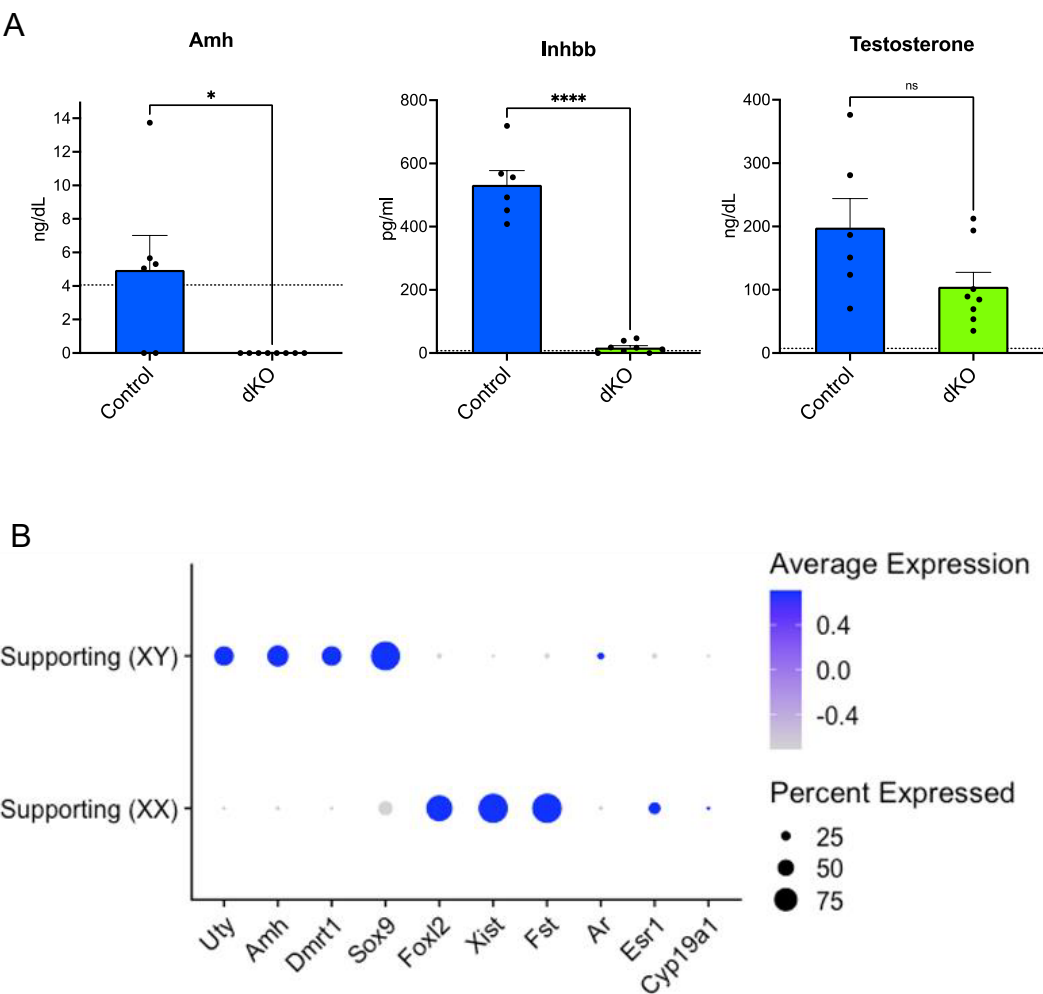
Supplemental Figure 4: Single cell analysis of control XX (ovary), control XY (testis) and dKO XY (ovotestis) at E15.5. (A) Dot plot of the gene expression of TGF-beta ligands and receptors in the different clusters. (B) UMAP distribution of the gene expression of the TGF-beta ligands: *Inhba*, *Bmp2*, *Bmp4* and *Tgfb2*. N= 2 independent biological replicates per genotype.

Supplemental Figure 5



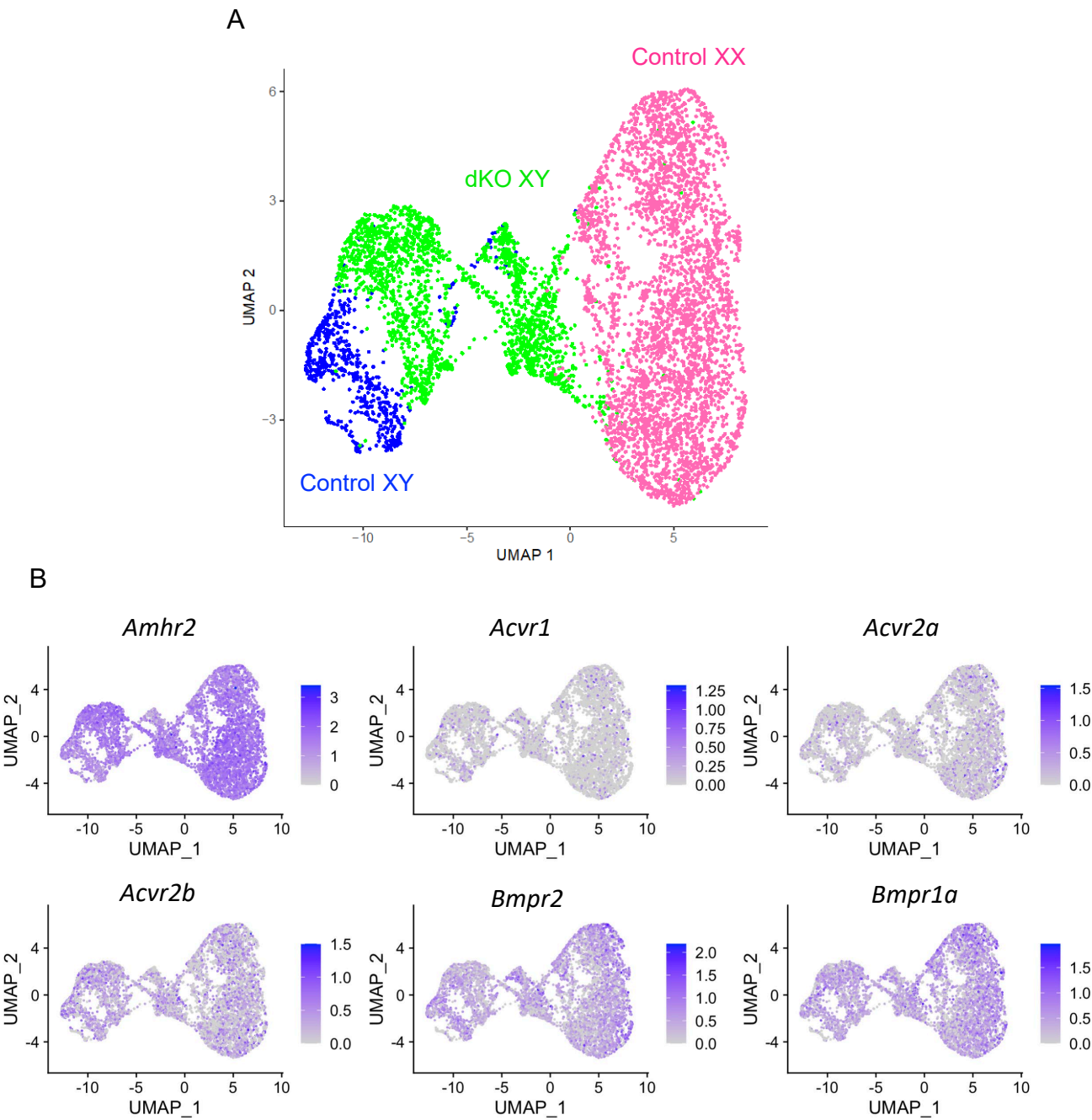
Supplemental Figure 5: Clustering of the supporting cells from control XY, dKO XY, and control XX gonads at E15.5. (A) Distribution on the UMAP of the female cell marker *Xist*, specific male supporting cell gene expression (*Amh*, *Dhh*, *Cst9*) and female supporting cell markers (*FoxL2*, *Rspo1*, *Fst*, *Runx1*). (B) Dot plots of genes used to identify each cluster. N= 2 biological replicates per group.

Supplemental Figure 6



Supplemental Figure 6: Serum levels of AMH, activin B, and testosterone in control XY (n=6) and dKO XY (n=8) adult mice (Source data are provided as a Source Data file; two-tailed T-test, * p= 0.01, **** p<0.0001) Bars depict average +/- SE. The dotted line shows the detection limit of the assay. (B) Dot plots of genes expressed in XY and XX supporting cells based on the single cell RNAseq dataset.

Supplemental Figure 7



Supplemental Figure 7: (A) Single cell mRNA sequencing analysis and clustering of the supporting cells based on their genotypes: control XY (blue), dKO XY (green), and control XX (pink) gonads at E15.5. (B) Expression of receptors for AMH (*Amhr2*, *Bmpr2*, *Bmpr1a*) and activins (*Acvr1*, *Acvr2a*, *Acrr2b*) over the UMAP(A).