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Supplementary Information for

Sexual fate of murine external genitalia development: conserved transcriptional competency for male-biased genes in both sexes.

Authors;

Daiki Kajioka[1†], Kentaro Suzuki[1†*], Shoko Matsushita[1], Shinjiro Hino[2], Tetsuya Sato[3], Shuji Takada[4], Kyoichi Isono[5], Toru Takeo[6], Mizuki Kajimoto[1], Naomi Nakagata[7], Mitsuyoshi Nakao[2], Mikita Suyama[3], Tony DeFalco[8], Shinichi Miyagawa[9] and Gen Yamada[1*]

* Correspondence: Gen Yamada, Ph.D., Kentaro Suzuki, Ph.D. Department of Developmental Genetics, Institute of Advanced Medicine, Wakayama Medical University, 811–1 Kimiidera, Wakayama 641–8509, Japan.

Email: transg8@wakayama-med.ac.jp (Y.G.), genyama778899@gmail.com (Y.G.), k-suzuki@wakayama-med.ac.jp (K.S.)

This PDF file includes:

Figures S1 to S5

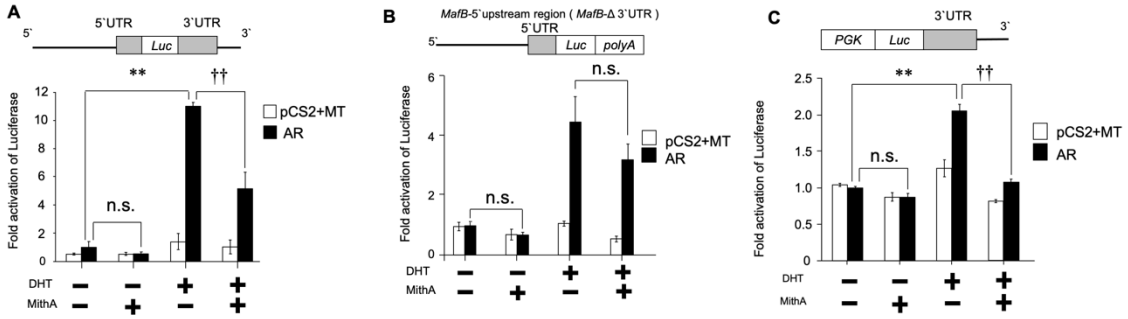

Fig. S1. Inhibition of Sp1 reduces AR regulatory *MafB* expression through its enhancer.

(**A-C**) Reporter vectors for luciferase assays were constructed as described in our previous report. Six hours after transfecting plasmid vectors in HepG2 cells, 100nM MithA was pretreated for 24 hours. (**A**) 100nM MithA inhibited DHT-AR induced luciferase reporter activity. (**B, C**) Effect of 100nM MithA on luciferase activity of *MafB*-5`upstream region and pmirGLO-*MafB*-3`UTR reporter assays. Three-nine independent biological replicates were performed. ** $p < 0.01$, †† $p < 0.01$, n.s. (not significant, $p > 0.05$).

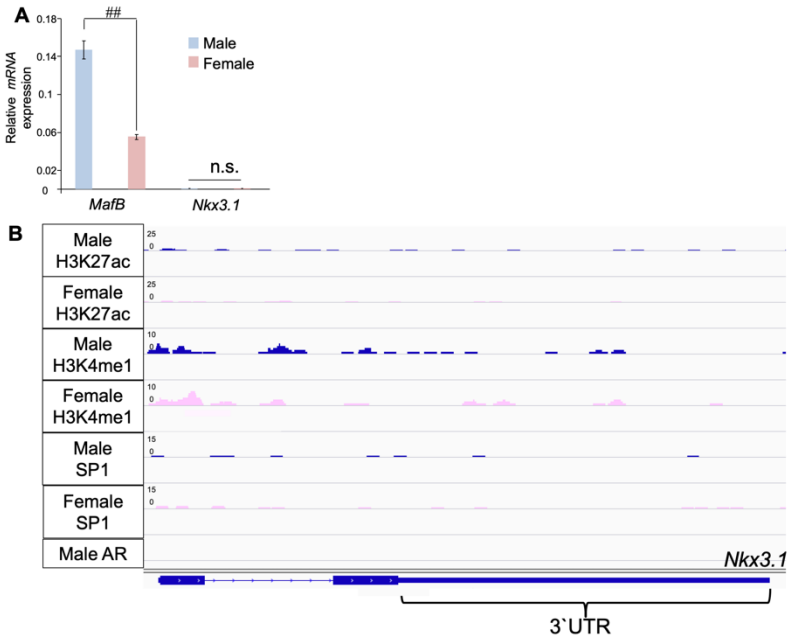

Fig. S2. *Nkx3.1* is not an androgen-regulated sex-biased gene in the eExG.

(**A**) Quantitative PCR of mRNA extracted from eExG urethral bilateral regions was performed to detect the expression levels of *MafB* and *Nkx3.1* normalized to levels of *Gapdh*. Three-five independent biological replicates were performed. ## $p < 0.01$, n.s. (not significant, $p > 0.05$). (**B**) Integrated Genomics Viewer (IGV) screenshot of ChIP-seq genomic tracks at the *Nkx3.1* gene locus. Active histone modifications, SP1, and AR were not detected in the eExG of both sexes.

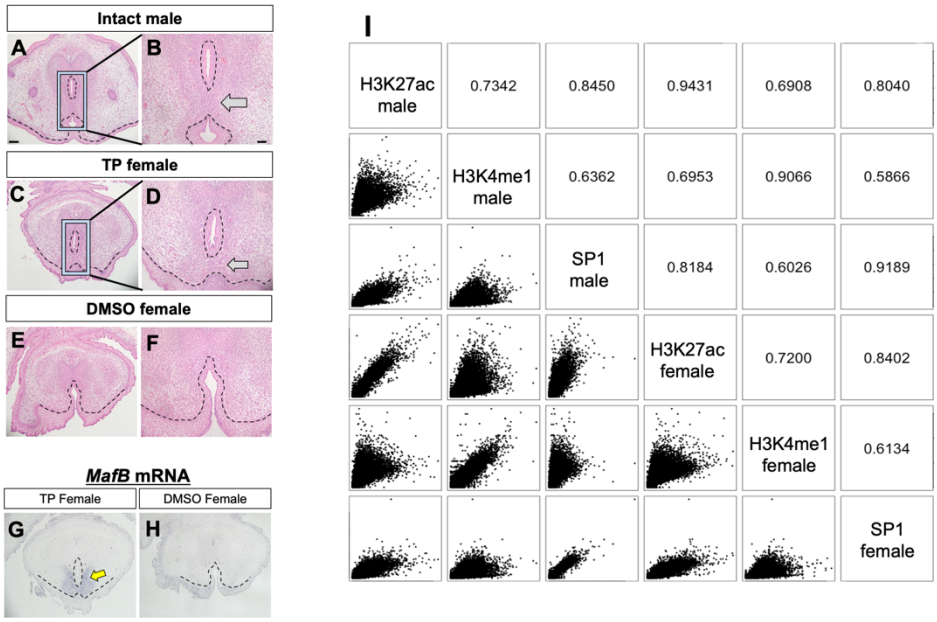

Fig. S3. Exogenous androgen induces male-type urethral differentiation in females.
(**A-F**) H&E staining for intact male eExG, TP- and DMSO-treated female eExG at E16.5. Gray arrows show male-type urethral differentiation. TP, testosterone propionate. (**G, H**) Section in situ hybridization for *MafB* mRNA for TP- and DMSO-treated female E16.5 eExG. Yellow arrow represents male-type urethral differentiation associated with *MafB* expression. Administration of TP or DMSO was started at E15.5 and embryos were harvested at E16.5. (**I**) Coefficient plots show that SP1 binding events were highly positively correlated with the presence of H3K27ac at AR-mediated regulatory elements. Dashed lines, epithelial-mesenchymal border. Scale bar in **A**, **C**, **E**, **G**, **H** = 100μm, Scale bar in **B**, **D**, **F** = 50μm.

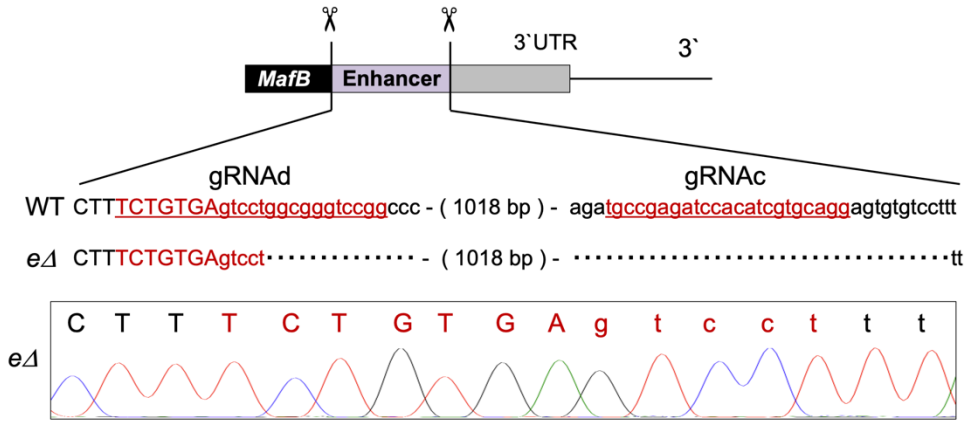

Fig. S4. Generation of *MafB* enhancer deletion mouse by the CRISPR-Cas9 genome editing system.

Track shows results of direct sequencing from *MafB* enhancer mutant mouse line. Red characters indicate target sequences by gRNAs. Large characters indicate coding sequences.

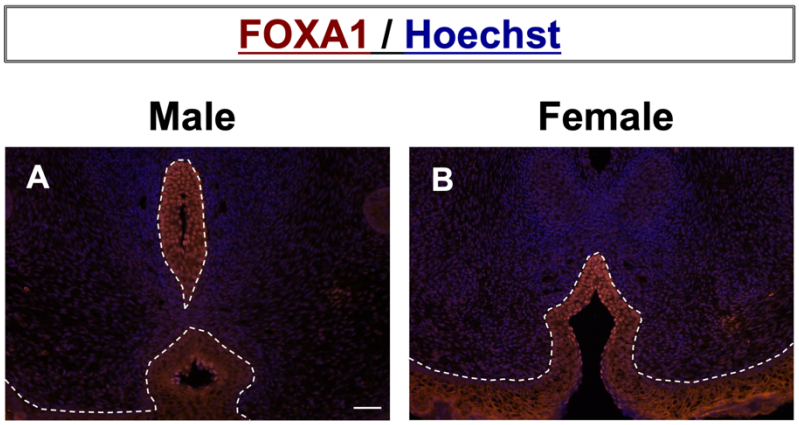

Fig. S5. Expression of FOXA1 in male and female eExG at E16.5.
(**A, B**) FOXA1 was predominantly expressed in the eExG endodermal epithelium. Scale bar, 50μm. Dashed lines, epithelial-mesenchymal border.