Supplemental Materials and Methods

Generation of the Rnase10 Targeting Vector. Homologous recombinogenic regions were derived from the 56J21 BAC identified by screening the RPCI-22 129/Sv mouse BAC library (Invitrogen) with a [^{32}P]-labeled *Rnase10* cDNA probe. An 8.8 kb genomic locus was captured by means of λ-mediated homologous recombination (Red/ET Recombination System, Gene Bridges GmbH, Heidelberg, Germany) with a gapped pACYC177 (GenBank Accession X06402) plasmid vector prepared by PCR with the following pair of oligonucleotides incorporating an *Not*I restriction site at either end (underlined): 5'-CTCCTACATG TGGTGAACTA AACCAGTGAG GTCACCGTGT CCAATAGATG GCGGCCGCTT CTTAGACGTC AGGTGGCAC-3' and 5'-GAATGCCCCT CATAAAATCA TATATTAAAT GTTTGGTCAC CAGGAAATAG GCGGCCGCGC GCTAGCGGAG TGTATACTG-3'. The vector core sequence was assembled within the pBluescriptSK(-) phagemid (Stratagene; GeneBank Accession X52330), in which the *Xho*I—*Hin*dIII region had been replaced with an inversely orientated *iCre* sequence (GeneBank Accession AY056050). The polyadenylation signal derived from the bovine growth hormone gene was amplified by PCR from the pKONeo plasmid (positions 3167-3379 in GeneBank Accession AF090454) adding an FRT sequence at the 3'-end, and subcloned between *Kpn*I and *Ngo*MIV restriction sites to follow *iCre*. An aminoglycoside-3'-O-phosphotransferase (Neo^{R}) expression cassette containing the *phosphoglucokinase* (*PGK*) gene promoter and a polyadenylation signal was derived from the *loxP-PGK-Tn5-neo-loxP* plasmid (Gene Bridges GmbH, Heidelberg, Germany) and added by means of λ-mediated homologous recombination using the following oligonucleotides: 5'-CATGCTGGGG ATGCGGGAAG TTCCTATACT TTCTAGAGAA TAGGAACTTC TTTTTCCCAA GGCAGTCTGG AG-3' and 5'-TTTCCCCGAA AAGTGCCACC TGGGACGCGC CCTGTAGCGG CGCATTAAGC AAGTTATACG CCAAGCTGG C-3'. The core sequence was targeted into the captured 8.8 kb genomic fragment so as to replace the first eight nucleotides of *Rnase10* exon 2; the following oligonucleotides incorporating an FRT site at the 3' end of the Neo^{R} cassette (underlined) were used: 5'-GGCCAGAGTT TATCTCTACA TCACGCCTTC ATTTTCCTCT TCTTCTTCAG TGTCCACCAT GGTGCCCAAG-3' and 5'-CCTAGCAACA GCAGCAACAT CATGAACAAC AGATGCACCA GTGTCACCTT GAAGTTCCTA TTCTCTAGAA AGTATAGGAA CTTCCGCCG CACACAAAAA CCAAC-3'. Error-free recombinants were identified by sequencing the insert throughout.

Gene Targeting in ES Cells and Derivation of Rnase10^{Cre} Mice. AB2.2 ES cells (Lexicon Genetics Inc., The Woodlands, TX) derived from 129S7/SvEvBrd-*Hprt*$^{b-m2}$ were grown on layers of neomycin-resistant primary mouse embryonic fibroblasts in Dulbecco's Modified Eagle's Medium supplemented with 15% ES cell-tested fetal bovine serum, non-essential amino acids (all from Invitrogen), 50 μM 2-mercaptoethanol and 1,600 units/ml murine leukemia inhibitory factor (ESGRO, Millipore, Billerica, MA) in a humidified atmosphere of 5% carbon dioxide in air. The

targeting construct was liberated from the plasmid vector with *Not*I restriction enzyme, and approximately 100 μg DNA at a concentration of 25 μg/ml was used for electroporation into ES cells prepared in PBS at a density of 1.1×10^7 cells/ml. Neo-resistant clones were selected over a period of 5-7 days in the presence of geneticin (Invitrogen) at a concentration of 350 μg/ml, and then grown for a further 5-10 days without geneticin until drug-resistant colonies reached 1.5-2 mm in diameter. At this point, individual clones were transferred to 96-well plates. Initial screening was carried out by PCR with a pair of primers amplifying a 2.25 kb 5' crossing-over product as shown in Fig. 1*A* (forward: 5'-GCAGTTAGTT TAGCAGGGAG-3', reverse: 5'-AGGTTTTGGT GCACAGTCAG-3'). Correct recombination events were confirmed by Southern blot hybridization of *Nhe*I/*Bst*ZI-digested ES cell DNA (Fig. 1*B*). To confirm euploidy, mitotic chromosomes were prepared from exponentially growing ES cells arrested with colcemid (2), and chromosome counting was carried out on at least 50 separate spreads. An ES cell clone with 92% euploid cells was used for microinjection into C57Bl/6 blastocyst in order to produce allophenic mice. Male chimeras were mated to C57Bl/6 females, and agouti progeny were genotyped to identify carriers of the modified allele.

Southern Blot Hybridization. ES cells were lysed with SDS/Proteinase K and DNA was extracted with isopropanol. After digestion with restriction enzymes, DNA fragments were electrophoretically resolved in agarose gels and transferred onto nylon membranes under alkaline conditions. Hybridization to [^{32}P]-labeled DNA probes was carried out as described (3). Ready-To-Go DNA Labeling Beads and [α-^{32}P]-dCTP (GE Healthcare, Buckinghamshire, UK) were used for the generation of probes (random primed labeling); templates were prepared by PCR utilizing the following primers: 5' probe: 5'-CAAAGACAAG GAGCAATGGG-3' and 5'-GACGCATGCT TTCTGTAGTC-3'; Neo probe: 5'-AGGATCTCCT GTCATCTCAC CTTGCTCCTG-3' and 5'-AAGAACTCGT CAAGAAGGCG ATAGAAGGCG-3'; 3' probe: 5'-ACTGCCTGAA AACAAGTTGG-3' and 5'-GGTAACATTA AAGGTAGGGG-3'.

Immonohistochemistry of EAAC1. After deparaffinization, the tissue sections were rehydrated and subjected antigen retrieval for 20 min at 80°C in 0.05 M glycine buffer, followed by 3 washes in Tris-buffered saline (TBS; 0.15 M NaCl, 0.05 M Tris/HCl pH 7.6). Non-specific binding was blocked by incubation with 5% (v/v) normal rabbit serum for 20 min at room temperature. Sections were then incubated for 1 h at room temperature with a polyclonal goat antibody against a synthetic peptide corresponding to amino acids 504-523 from the carboxy terminus of the cloned rat EAAC1 (1:400, Millipore, UK). After 3 washes in TBS, the sections were incubated with biotinylated rabbit anti-goat immunoglobulin (Ig) G (1:1000, Vectorlabs, UK) for 1 h at room temperature, followed by 3 further washes in TBS and incubation with alkaline phosphatase conjugated to extravidin (Sigma, UK) for 1 h at room temperature. All antibody buffers contained 1% (w/v) bovine serum albumin (BSA, Sigma). For visualization, slides were placed in solution containing 0.35% of nitro blue tetrazolium and 5-

bromo-4-chloro-3-indolynitrolphosphate (Roche Applied Sciences, UK) for 10 min at room temperature. Sections were counterstained for 30 sec with Mayers hematoxylin and mounted in glycerol gelatin mountant (Sigma, UK).

References

1. **Zhang Y, Muyrers JP, Testa G, Stewart AF** 2000 DNA cloning by homologous recombination in Escherichia coli. Nat Biotechnol 18:1314-1317

2. **Akeson EC, Davisson MT** 2003 Analysing mouse chromosomal rearrangements with G-banded chromosomes. In Jackson IJ, Abbott CM, eds. Mouse Genetics and Transgenics: A Practical Approach. Oxford, UK: Oxford University Press; 144-153

3. **Sambrook J, Fritsch EF, Maniatis T** 1989 Molecular cloning: a laboratory manual, 2nd Edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press

Supplemental Table 1. Oligonucleotide primers used in quantitative real-time RT-PCR analysis.

Gene (***Symbol***, name; MGI ID)	**Oligonucleotide pair**	**Conc., nM**
Rnase10*,* ribonuclease, RNase A family, 10; MGI:1922269	5'-TAGGAGAGCAGAACTGGGGA-3' 5'-ATGCACCAGTGTCACCTTCA-3'	300 900
Ros1*,* Ros1 proto-oncogene; MGI:97999	5'-AGCTGCCTAACGTCCTGTGT-3' 5'-AGCTGCCTAACGTCCTGTGT-3'	500 500
Etv4*,* ets variant gene 4 (E1A enhancer binding protein, E1AF), MGI:99423	5'-AAACAGGAGCGCACAGACTT-3' 5'-GGAATGGTCGAAGGGATTTT-3'	500 500
Araf*,* v-raf murine sarcoma 3611 viral oncogene homolog; MGI:88065	5'-AGTTCCACCAGCATTGTTCC-3' 5'-GGGGTTAGCAGCTCATTCAC-3'	500 500
Srd5a1*,* steroid 5 alpha-reductase 1; MGI:98400	5'-CCAGGGGAAACTGGATACAA-3' 5'-CACAGGGTGAACAGAGCAAA-3'	500 500
Bcl2l15*,* BCLl2-like 15; MGI:2685412	5'-CTGCTAACCGGAACCTATCG-3' 5'-AAGCTTCCAGCTCTCCATTG-3'	500 500
Gapdh*,* glyceraldehyde-3-phosphate dehydrogenase; MGI:9564	5'-AAGGGCTCATGACCACAGTC-3' 5'-GGATGCAGGGATGATGTTCT-3'	300 900

Supplemental Fig. 1. Zone of *Ar* ablation in proximal epididymis of ProxE-ARKO male at day 35 post partum. Immunohistochemical detection of androgen receptor protein, counter-staining with hematoxylin. Arrow indicates the segment of epididymal tubule displaying mosaicism (AR-positive and AR negative principal cells). Bar = 500 μm.

Supplemental Fig. 2. *Ar* inactivation in ProxE-ARKO males occurring in principal cells concurrently with differentiation of epithelial cell types in the proximal epididymal duct. Shown are progressive changes in the proximal segment of epididymides from WT *(top)* and ProxE-ARKO males *(bottom)* during the pre-pubertal period (immunohistochemical staining with anti-AR antibody). On days 20-22 post partum, the epididymal duct is lined with simple cuboidal epithelium *(left)*. Between days 20 and 25 of life, pseudostratification of the epithelium is observed, with proliferation and differentiation of the main epithelial cell types (*A/N*, apical/narrow cells with adluminally positioned nuclei; *B*, basal cells, typically distinguishable by hemispherical nuclei lying on the basal membrane; *P*, numerous principal cells with nuclei occupying the middle row). In ProxE-ARKO epididymides AR-negative cells are detectable during pseudostratification of the epithelium, and in peri-pubertal specimens (30-35 dpp) *Ar* ablation is restricted to principal cells. Bar = 30 μm.

Supplemental Fig. 3. Immunohistochemical detection of EACC1 protein in the epididymis of WT and ProxE-ARKO mice. At 40 dpp: initial segment (*c*, *d*); corpus epididymis (*e*, *f*); cauda epididymis (*g*, *h*) in WT (*a*, *c*, *e*, *g*) and ProxE-ARKO (*b, d, f, h*) mice. Panels *a* and *b* depict the negative control (minus primary antibody) of WT and ProxE-ARKO mice respectively. Bar = 50 μm (counter-staining with hematoxylin).

Supplemental Fig. 4. Immunohistochemical detection of AR protein in reproductive organs of WT mice at 40 dpp. *a*, testis (*S*, Sertoli cells; *L*, Leydig cells); *b*, efferent ducts; *c*-*i*, epididymis segments IV-X; *j*, vas deferens; *k*, seminal vesicle; *l*, coagulating gland; *m*, ampullary gland; *n*, ventral prostate; *o*, dorsal prostate. Bar = 50 μm (counter-staining with hematoxylin).

Supplemental Fig. 5. Gradual appearance of the ProxE-ARKO phenotype in proximal epididymis. *A*, section of proximal epididymis in a 40 day-old wild type littermate. *B* and *C*, proximal epididymides of two knockout mice with differential progression of the phenotype: decrease in epithelial height with loss of supranuclear cytoplasm (*B*) and accumulation of sperm with progressive dilatation of the tubules (*C*); *e.d.*, efferent ducts. *D* shows more advanced tubule obstruction with further build up of spermatozoa and flattening of the epithelium. Bar = 200 μm; H&E.

Figure 1

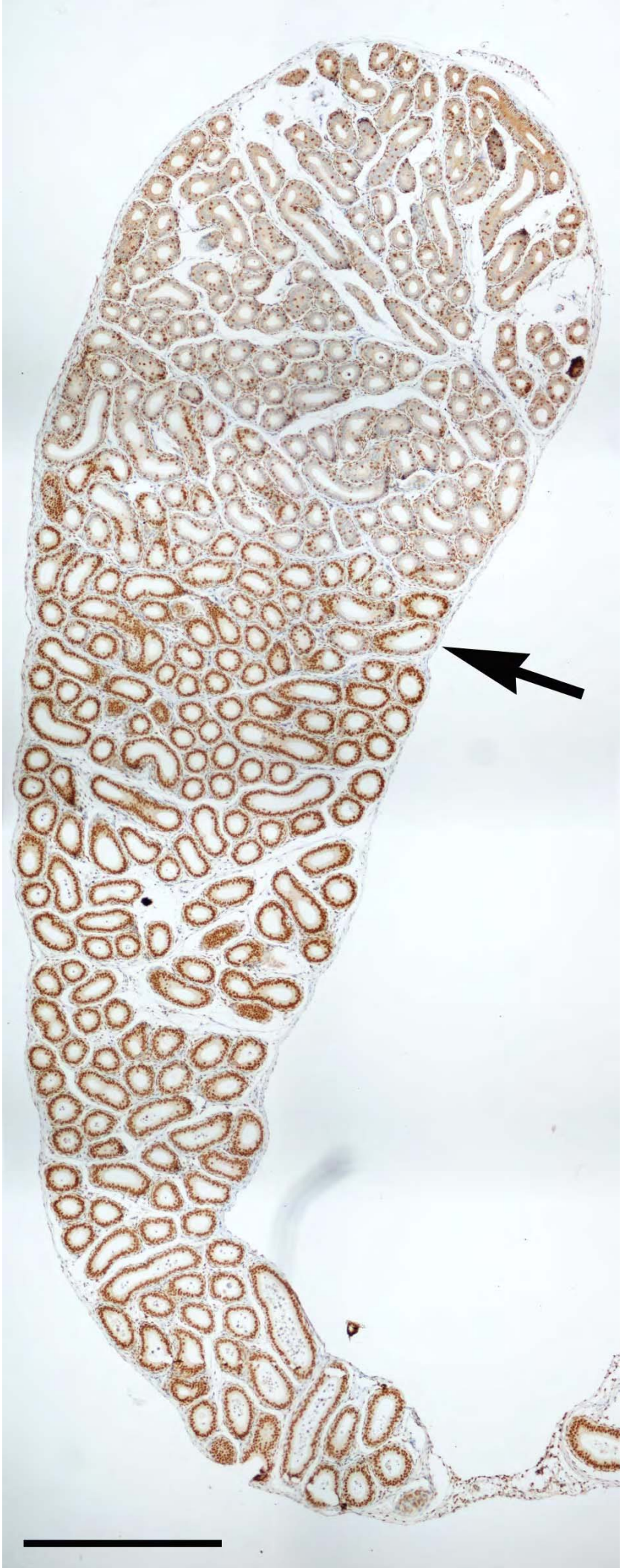
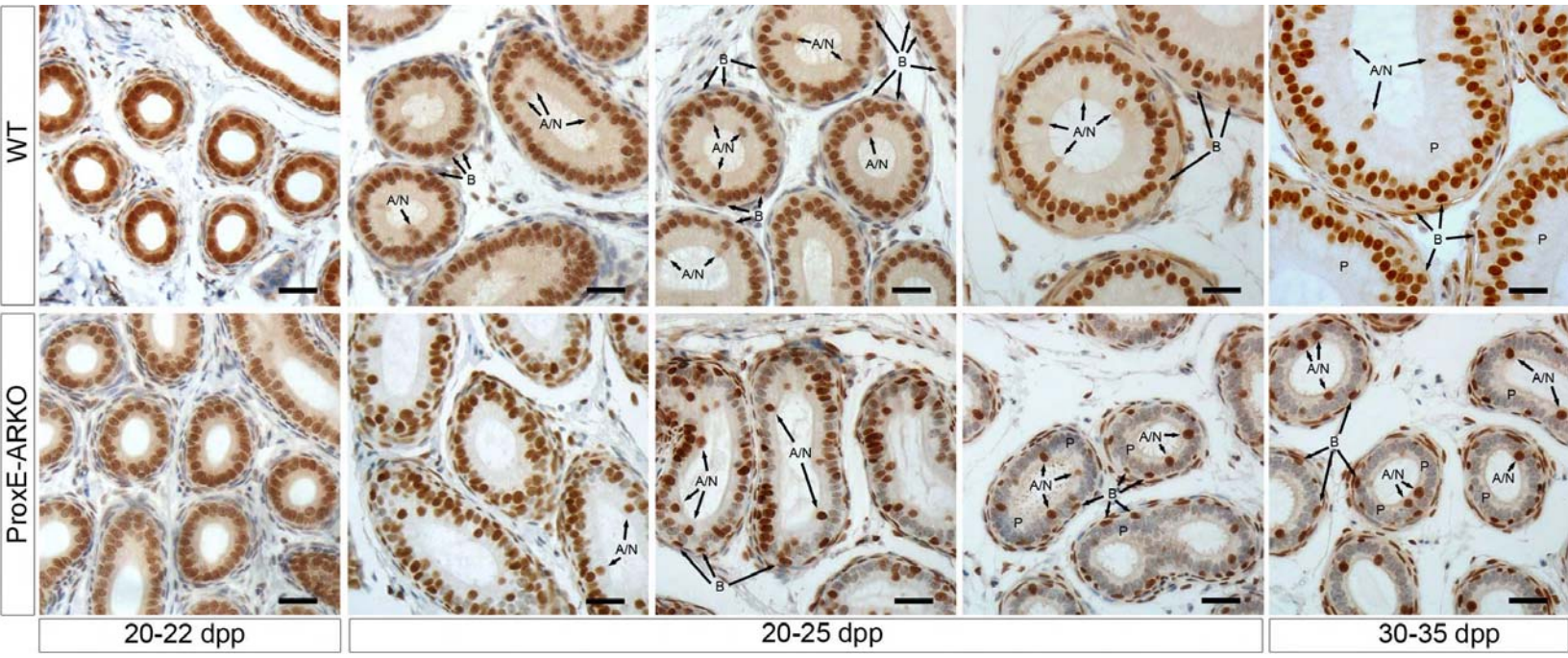


figure 2



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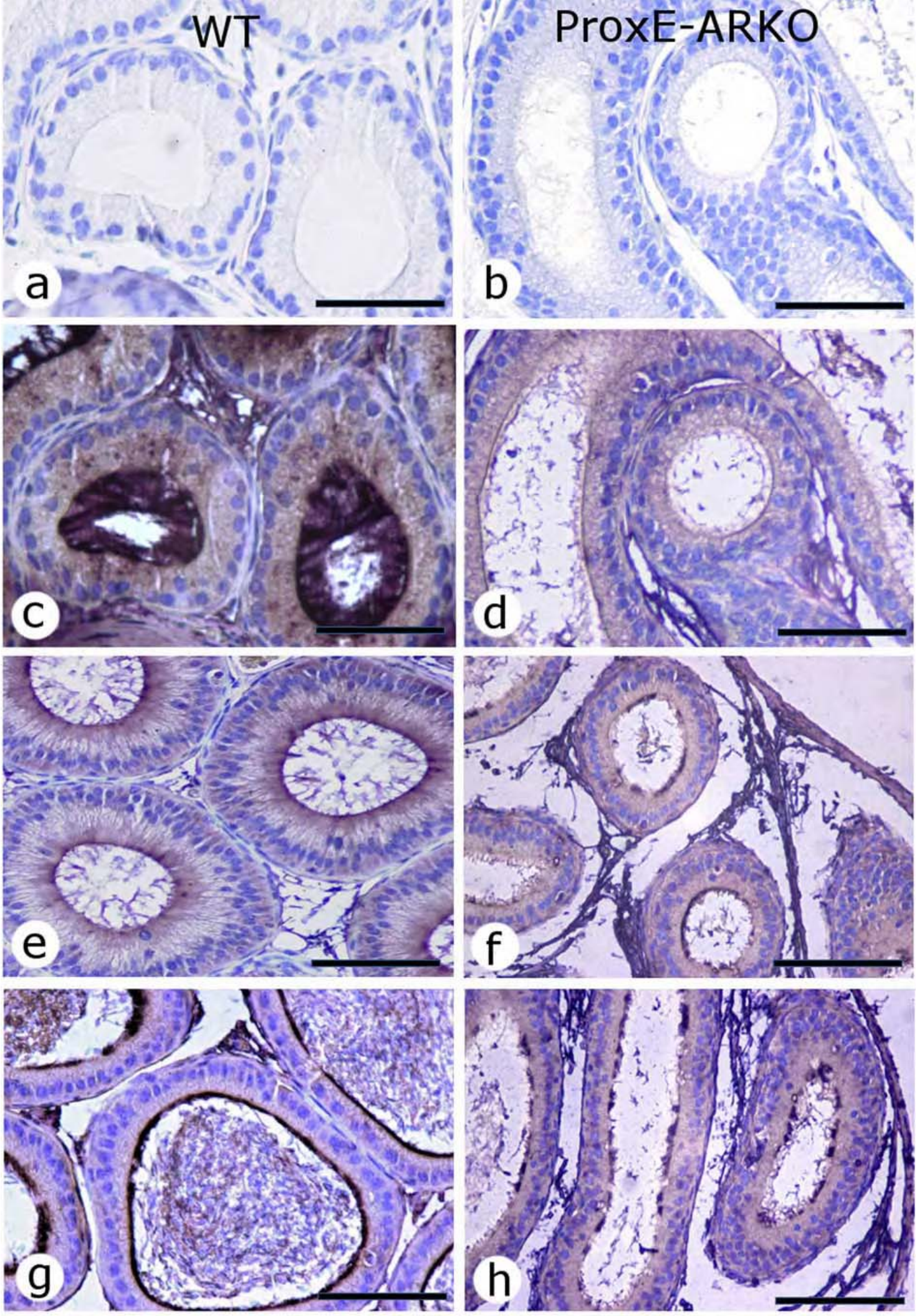

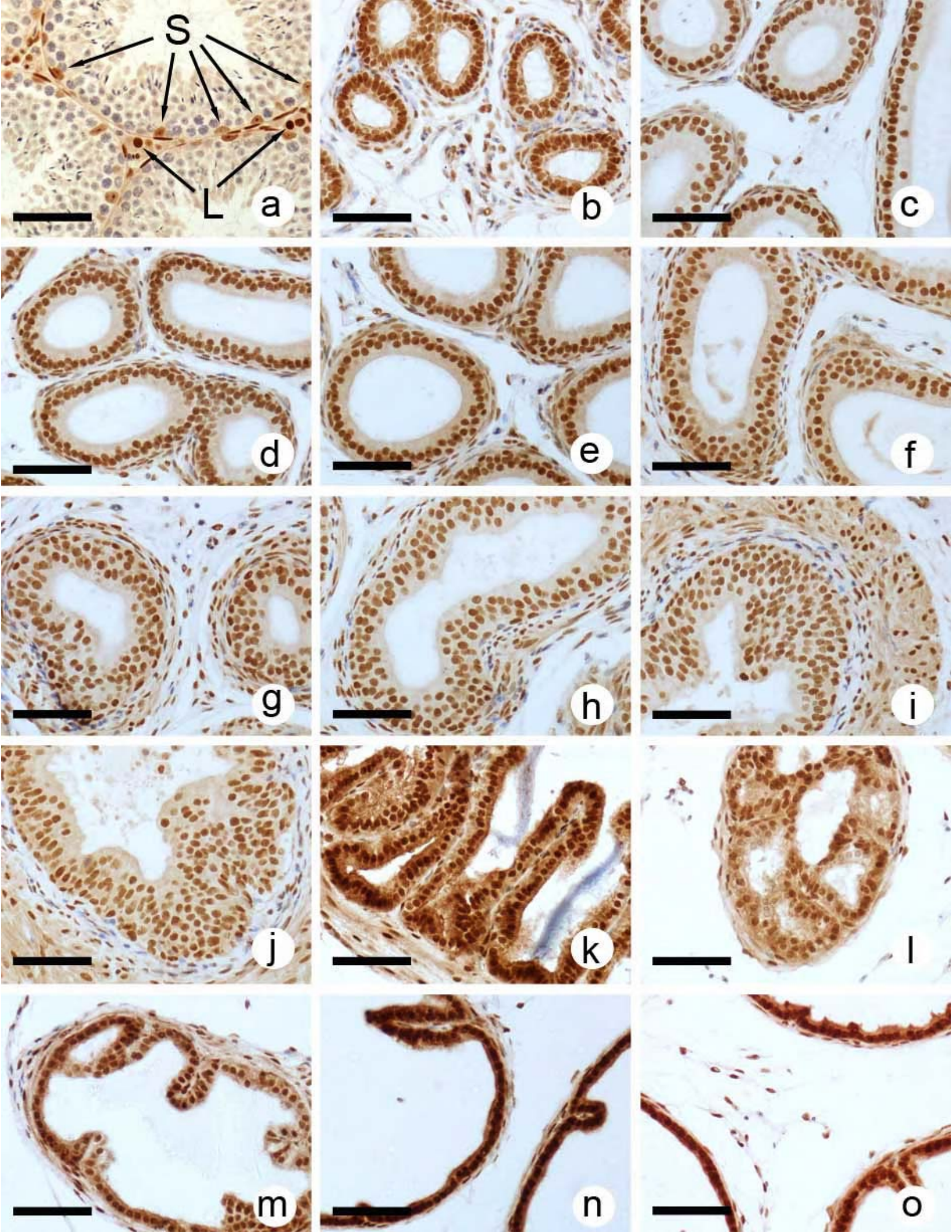
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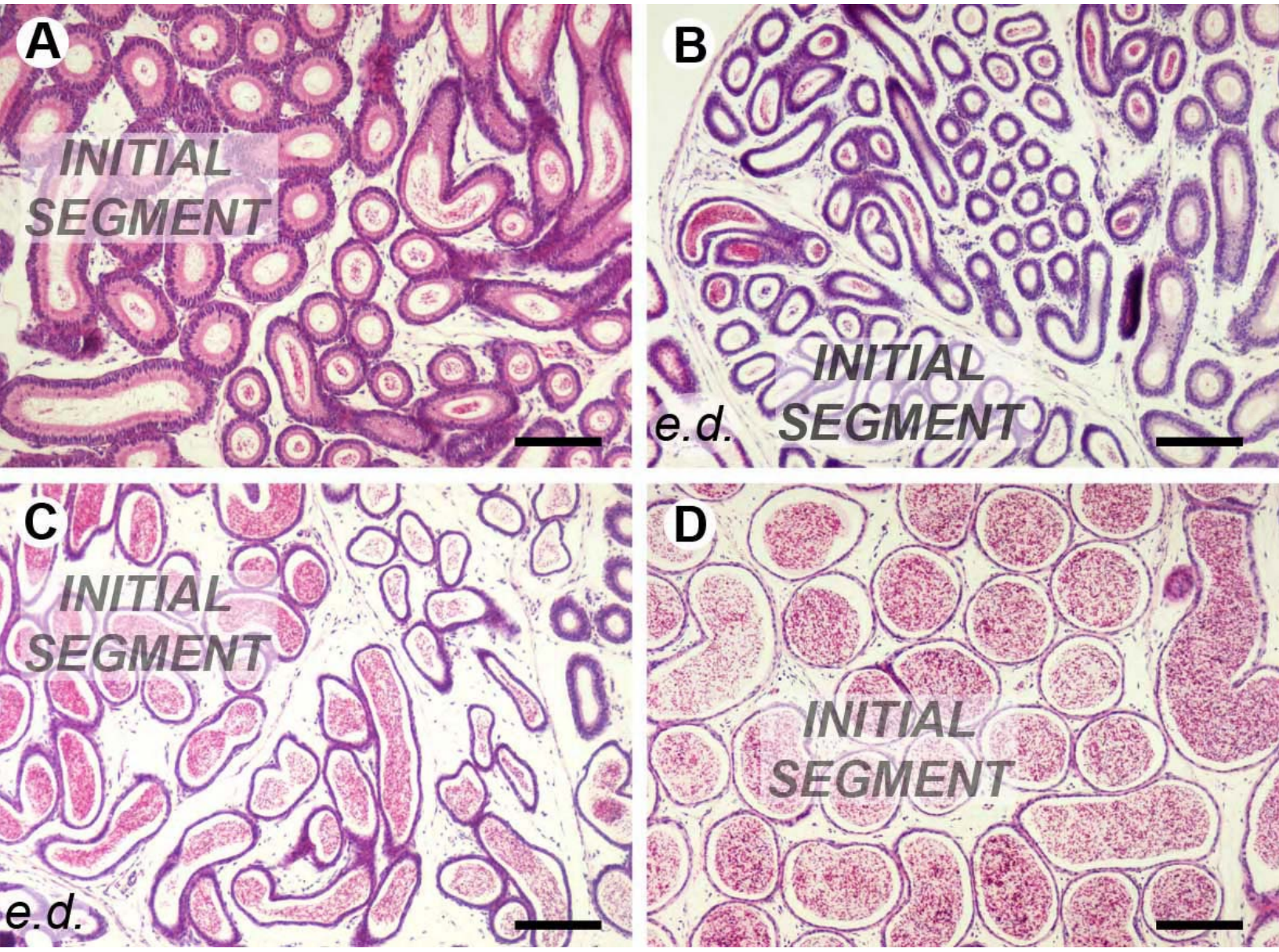
Figure 5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