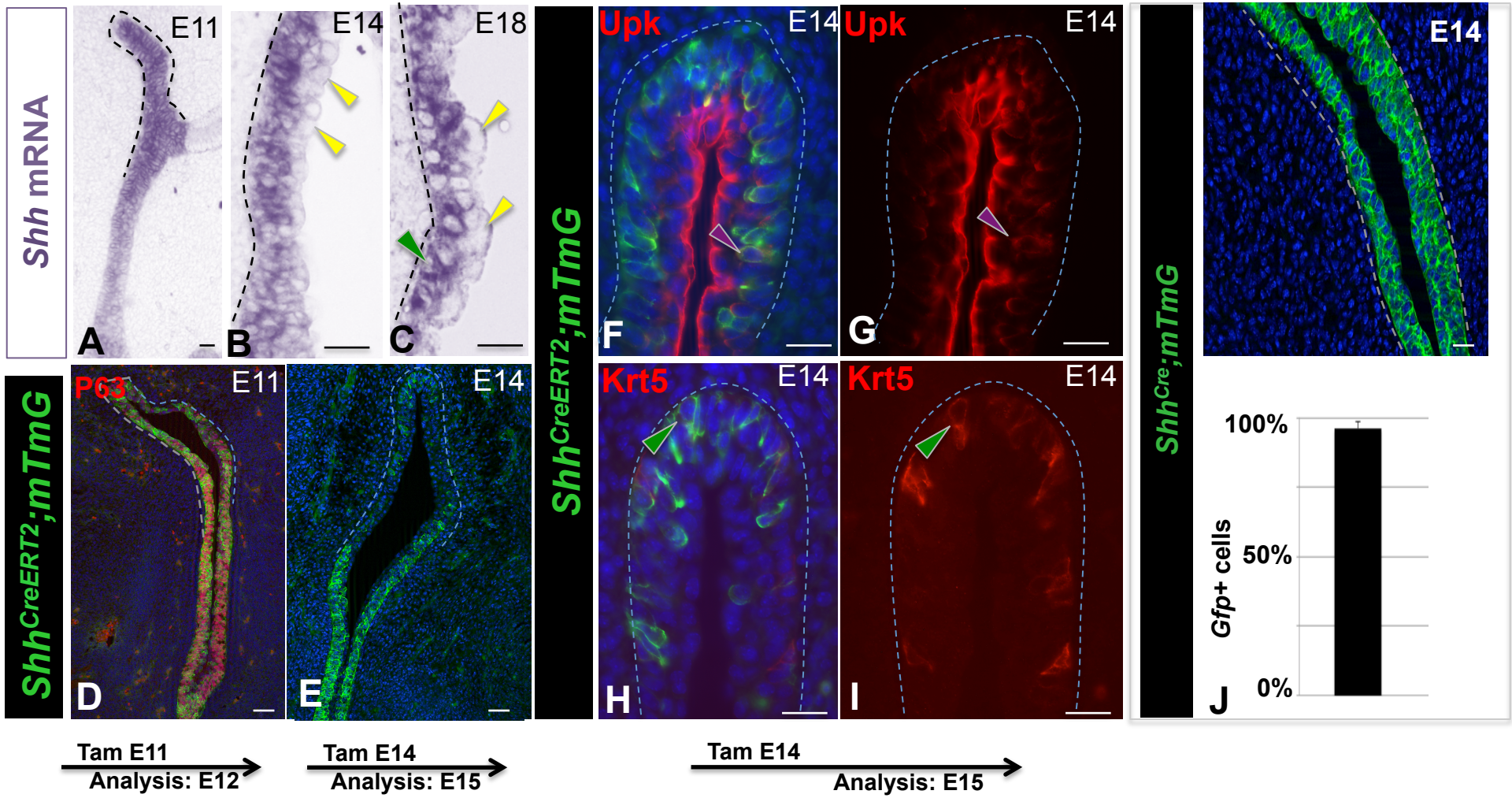
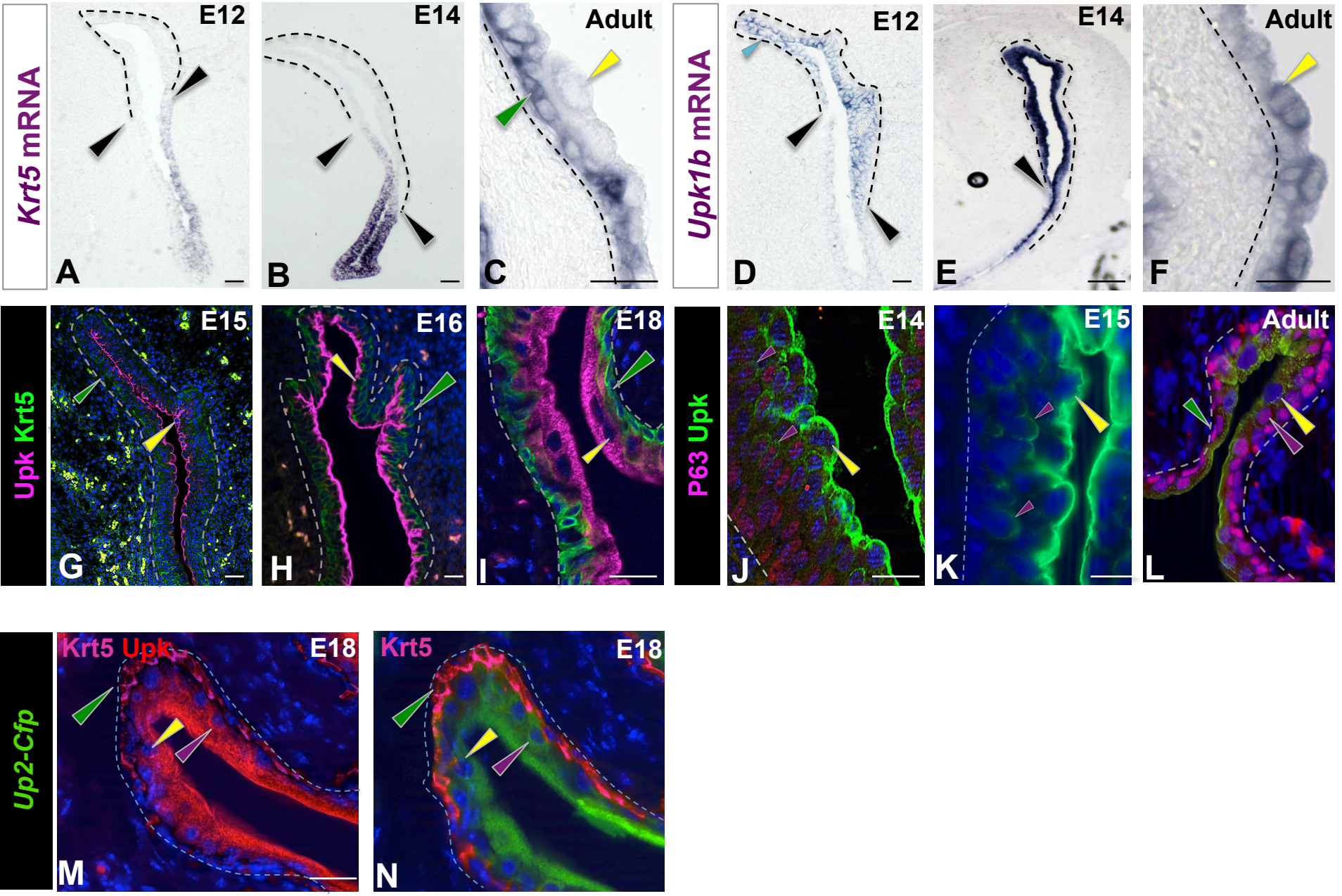
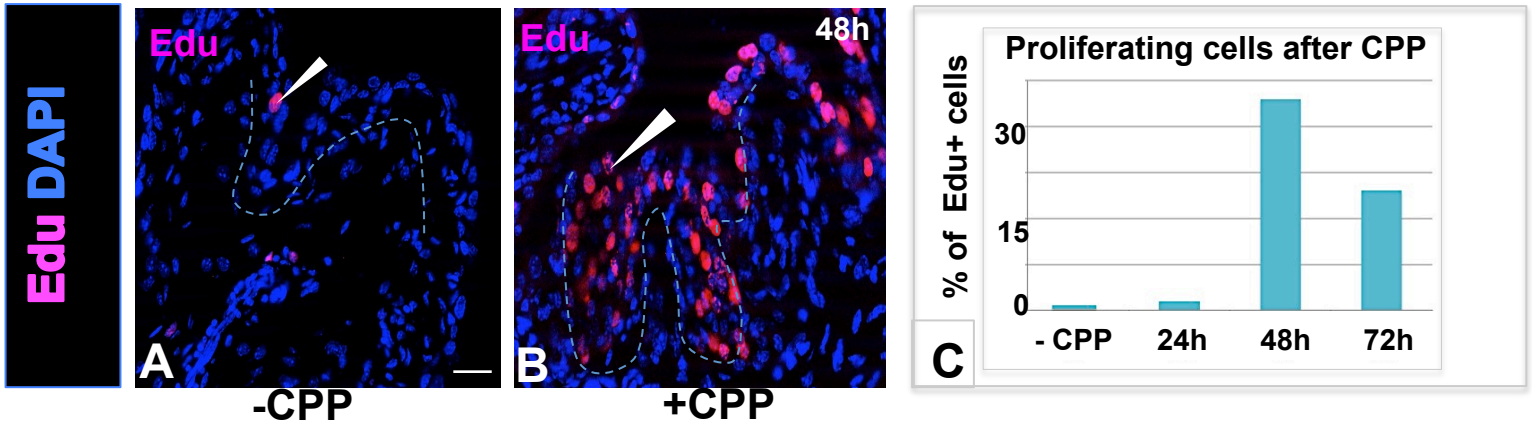


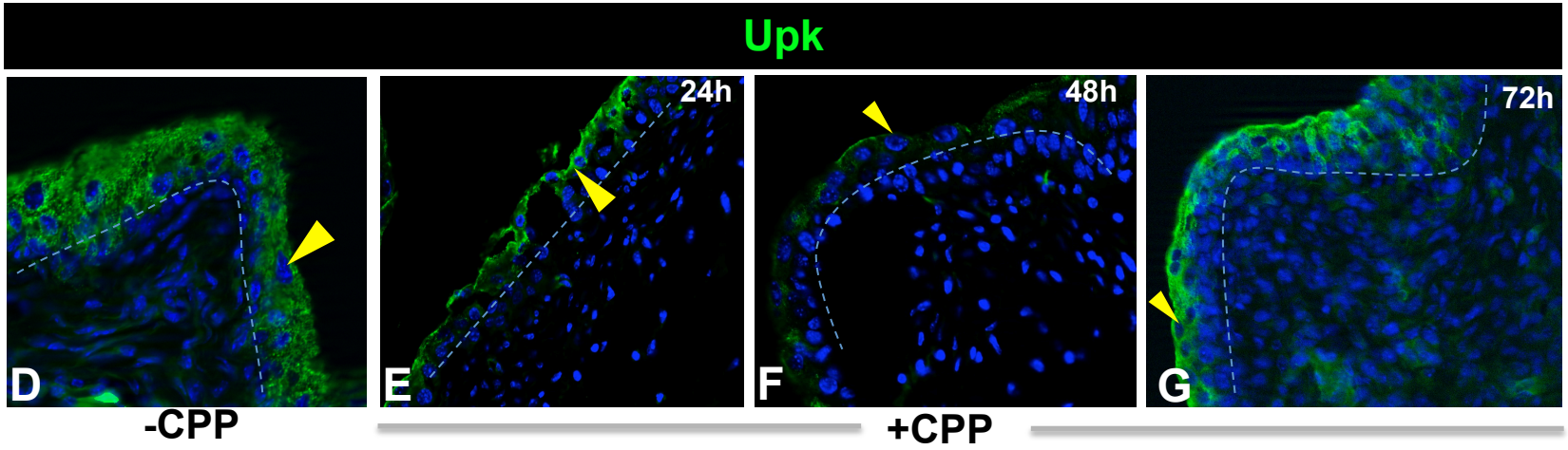
Supplementary Figure 1

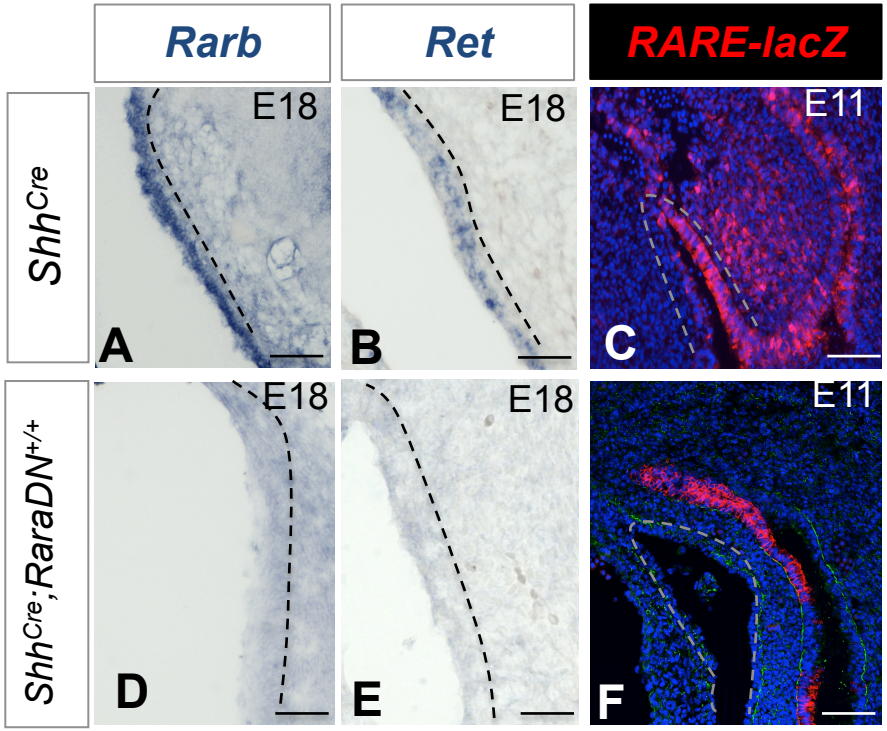


Supplementary Figure 2

Supplementary Figure 3







Supplementary Figure 4

Table S1. Phenotypes induced by expression of *RaraD*N phenocopy those in RA-deficiency and in mutants lacking components of the RA-signaling pathway.

	VAD [1-4]	RARKO [5-10]	Raldh2KO [9,11,12]	RaraDN [11,12]	PPAR TR VDR [13-15]
Kidney					
Renal agenesis	+	+	+	+	−
Renal hypoplasia	+	+	+	+	−
Down-regulation of Ret	+	+	+	+	−
Ureter/Wolffian duct					
Ectopic ureter insertion	+	+	+	+	−
Ectopic vas deferens	+	+	+	+	−
Hydronephrosis	+	+	+	+	−
Bladder/urethra					
Bladder hypoplasia	+	+	+	+	−
Keratinization of the urogenital sinus and its derivatives	+	+	**unknown**	+	−

1. Wilson and Warkany. 1948; 2. Wolbach and Howe, 1925; 3. Liang, et al., 2005; 4. Quadro et al., 2005; 5. Lohnes et al., 1993; 6. Mendelsohn et al., 1994; 7. Batourina et al., 2001; 8. Batourina et al., 2002, 9.Batourina et al., 2005; 10. Luo et al, 1996; 11. Chia et al., 2011; 12. Rosselot et al., 2010; 13. Barak et al., 1999; 14. Panda et al., 2001; 15. Gothe et al., 1999.

Legends-Supplementary Figures

Supplementary Figure S1 (related to Figure 1). **Analysis of the specificity of Cre-dependent recombination in *Shh^{CreERT2};mTmG* embryos.** A-C. In situ analysis showing expression of Shh mRNA at E11 (A), E14 (B) and E18 (C). D. P63 expression in a section from an E12 *Shh^{CreERT2};mTmG* embryo exposed to TM on E11. E A section from an E15 *Shh^{CreERT2};mTmG* embryo exposed to TM on E14. F. Upk expression in a sectioned urothelium from an E15 *Shh^{CreERT2};mTmG* embryo exposed to TM on E14. G. Same section as in F., showing only the Red channel (Upk-expression). H. Krt5-expression in an E15 *Shh^{CreERT2};mTmG* embryo exposed to TM on E14. For quantification, a minimum of three independent experiments were performed, and the average ± SEM was plotted. **Magnifications :** A,B 10X; C 40X; D,E 20X; F-I 40X; scale bars 50μm.

Supplementary Figure S2 (related to Figure 2). **Marker analysis of the developing urothelium**. A-C. In situ hybridization analysis showing Krt5 expression in an E12 embryo (A), an E14 embryo (B), and in an adult (C). D-F. In situ hybridization analysis showing expression of Upk1b in an E12 embryo (D), an E14 embryo (E), and in an adult (F). G-I. Sections of E15 (G), E16 (H) and E18 (I) embryos co-stained for expression of Upk and Krt5. J. Upk and P63 expression in a section from an E14 embryonic urothelium. K. Upk expression in a section from an E15 embryonic urothelium. L. Upk and P63 stained section from an adult urothelium. M-N. Specificity of *Cfp* expression in *Up2-Cfp* transgenic mice. M. Upk and Krt5 expression in an E18 **Up2-Cfp** embryo (shown without the green channel). N. *Up2-Cfp* expression in a serial section from the same embryo as in (M) co-stained with Krt5. **Magnifications:** A,B, G,H 10X; C 40X; J,K, 40X; I, M,L 20X scale bars 50μm;

Supplementary Figure 3. (related to Figure 3)**. The CPP-induced damage and repair model.** A. Edu labeling (pink) in a wild type adult not treated with CPP. B. Edu-labeled cells (pink) in an adult 48h after CPP administration. C. A chart showing the proportion of proliferating cells in the urothelium of untreated and CPP-treated adults. D. Upk expression (green) in the urothelium of an untreated wild type adult. E. Upk expression (green) 24h after CPP treatment. F. Upk expression (green) 48h after CPP treatment. G. Upk expression (green) 72h after CPP treatment. **Magnifications:** A,B,H,I 20X; D-G 40X. Scale bars 50μm.

Supplementary Figure 4. (related to Figure 7). **RA-signaling is down-regulated in the urothelium of *Shh^{Cre};RaraDN* mutants**. A. In situ hybridization analysis showing *Rarb* expression in the urothelium of an E18 *Shh$^{Cre/+}$* control embryo. B. In situ hybridization analysis showing *Ret* expression in the urothelium of an E18 *Shh$^{Cre/+}$* control embryo. C. RARE-lacZ activity in the urothelium of an *Shh$^{Cre/+}$;RARE-lacZ* E11 control embryo. D. In situ hybridization analysis showing lack of *Rarb* expression in the urothelium of an E18 *Shh^{Cre};RaraDN* mutant. E. In situ hybridization analysis showing lack of *Ret* expression in the urothelium of an E18 *Shh^{Cre};RaraDN* mutant. F. Down-regulated RA-reporter activity in the urothelium of an E11 *Shh^{Cre};RaraDN;RARE-lacZ* mutant embryo. **Magnifications**: A-F 10X scale bars 50μm

Supplementary Table 1. Expression of the RaraDN generates defects that phenocopy those in RA deficiency. Comparison of urinary tract phenotypes induced by dietary Vitamin-A deficiency, deletion of RA receptors and Raldh2 knockouts with those in mice expressing RaraDN driven by different cell-type-specific Cre lines compared to phenotypes observed in mutant lacking other nuclear receptor family members (PPAR, VDR and TR) that bind Rxr.

EXPERIMENTAL PROCEDURES

Mice used in this study and primers used for genotyping. Genotyping was done by PCR of the tail or yolk sac DNA using a DNA Thermal Cycler PTC-100 (BIO-RAD, Hercules, CA, USA) with 40 cycles of 94C for 30 seconds, 53.5C for 30 seconds and 72C for 40 seconds, except for *RaraDN*, where we performed 45 cycles of 94C for 30 seconds, 54.5C for 30 seconds and 72C for 40 seconds.

Primers: *RARE-LacZ* mice were genotyped using primers 5'-CGTCGTCCCCTCAAACTGGCAGATGC-3' (forward) and 5'-TTCGGCGCTCCACAGTTTCGGGTTTTC-3' (reverse) generating a 570 bp product. Primers for genotyping *RaraDN* mice were 5'-ATGGTGTACACGTGTCACC-3' (mutated forward), 5'-CACCTTCTCAATGAGCTCC-3' (mutated reverse), 5'-TGGCTCGTGTCAAAGAACTG-3' (wild-type forward) and 5'-TGGTCGGTAGAAAGGCAGAG-3' (wild-type reverse) to generate a 210 bp mutant and a 426 bp wild type bands. *Shh*^{Cre} and *Shh*$^{Cre\mathit{ERT2}}$ mice genotyped using the following primers: 5'-AGGTGGACCTGATCATGGAG-3' (forward) and 5'-ATACCGGAGATCATGCAAGC-3' (reverse) generating a 440 bp product. *Krt5*$^{\mathit{CreERT2}}$ mice were genotyped using primers 5'-ATTTGCCTGCATTACCGGTC-3' (forward) and 5'-ATCAACGTTTTGTTTTCGGA-3' (reverse) generating a 350 bp product. For genotyping Upk2-CFP mice we used primers 5'-CACTCCGAGACAAAATCAGCTACC-3' and 5'-CGTCGTCCTTGAAGAAGATGGT-3' generating a 450 bp product. Primers for Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J reporter were 5'-CTCTGCTGCCTCCTGGCTTCT-3' (forward) and 5'-TCAATGGGCGGGGGTCGTT-3' (reverse) generating a 250 bp product.

Allele	Name in text	Source	Ref
B6;129S6-Gt(Rosa)26Sor$^{\mathit{tm2(mCherry)Mgn}}$	*mCherry*	MMRC (stock # 036286-UCD)	(Chen et al., 2011)
Upk2-Cfp	*Up2-Cfp*	Mendelsohn lab, Columbia University, NY	(this study)
Shhtm1(EGFP/cre)Cjt/J	*Shh*Cre	Jackson Laboratory (stock #005623)	(Harfe et al., 2004)

Shhtm2(cre/ESR1)Cjt/J	*Shh*^{CreERT2}	Jackson Laboratory (stock #005623)	(Harfe et al., 2004)
Krt5^{CreERT2}	K5^{CreERT2}	Daniel Metzger, IGBMC, France	(Indra et al., 1999)
Rare-hsp68-lacZ	*RARE-lacZ*	Dr. Janet Rossant,	(Rossant et al., 1991)
Gt(ROSA)RARa403	*RaraDN*	Mendelsohn lab, Columbia University,	(Rosselot et al., 2010)
CMV/b-act-LoxP-Egfp-LoxP/nlsLacZ	T83-lacZ	Metzger Lab, IGBMC, France	Unpublished
Tg(Upk3a-GFP/cre/ERT2)26Amc/J	*UPK3aGCE*	McMahon Lab, Harvard University	www.GUDMAP.org
Foxa2^{CreERT2}	*Foxa2*^{CreERT2}	Jackson Laboratory (stock #008464)	(Frank et al., 2007)
Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J	R26*mGmT*	Jackson Laboratory (stock #007576)	(Luo et al., 1996; Muzumdar et al., 2007)

PRIMARY ANTIBODIES USED IN THIS STUDY				
Antigen	**Supplier**	**Ig Type**	**Dilution**	**Method**
Trp63	Santa Cruz (4A4): sc-8431	Mouse IgG	1:100	Paraffin
TRp63	Santa Cruz (H-137): sc-8343	Rabbit IgG	1:100	Cryosections
Keratin 5	Covance (AF 138): PRB-160P	Rabbit IgG	1:200	Paraffin, Cryosections
Pan Uroplakin	Dr. T. T. Sun NYU	Rabbit IgG	1:1000	Paraffin, Cryosections
LacZ	Biogenesis 4600-1409	Goat IgG	1:100	Paraffin, Cryosections

Click-it EdU Alexa Fluor Azide Kit	Invitrogen E10415			Paraffin
Cfp/Gfp	Rockland (600-101-215)	Goat IgG	1:100	Paraffin Cryosections
Foxa2	Seven Hills Bioreagents (WRAB-1200)	Rabbit IgG	1:1000	Paraffin

SECONDARY ANTIBODIES USED IN THIS STUDY				
Alexafluor 488 donkey anti-goat	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Alexafluor 488 donkey anti-rabbit	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Alexafluor 594 donkey anti-rabbit	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Alexafluor 594 donkey anti-mouse	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Alexafluor 488 goat anti-mouse	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Alexafluor 594 donkey anti-rat	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Alexafluor 594 donkey anti-goat	Invitrogen	IgG (H+L)	1:1000	Paraffin, Cryosection
Cy5-conjugated donkey anti-mouse	Jackson Immunoresearch	IgG (H+L)	1:500	Paraffin, Cryosection
Cy5-conjugated donkey anti-goat	Jackson Immunoresearch	IgG (H+L)	1:500	Paraffin, Cryosection
Cy5-conjugated donkey anti-rabbit	Jackson Immunoresearch	IgG (H+L)	1:500	Paraffin, Cryosection

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