

SUPPLEMENTAL DATA

Figure Suppl. 1

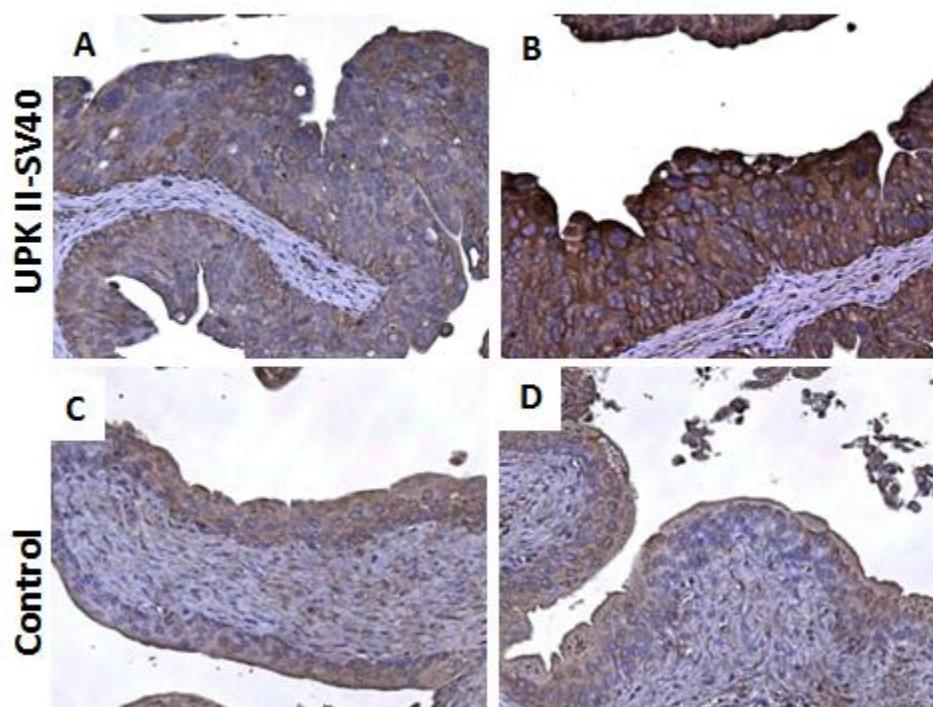


Figure Supplementary 1. Glut-1 expression in UPK II-SV40 bladder carcinoma *in situ*. A-D, immunohistochemistry for Glut-1, a glucose transporter whose expression is related to hypoxia, was performed in bladder tissues from UPK II-SV40 and control mice. Representative images are shown (200x original magnification), exhibiting an increased staining for Glut-1 in urothelial carcinoma *in situ* of UPK II-SV40 mice, with a pattern of staining almost exclusively located in the malignant epithelial cells.

Figure Suppl. 2

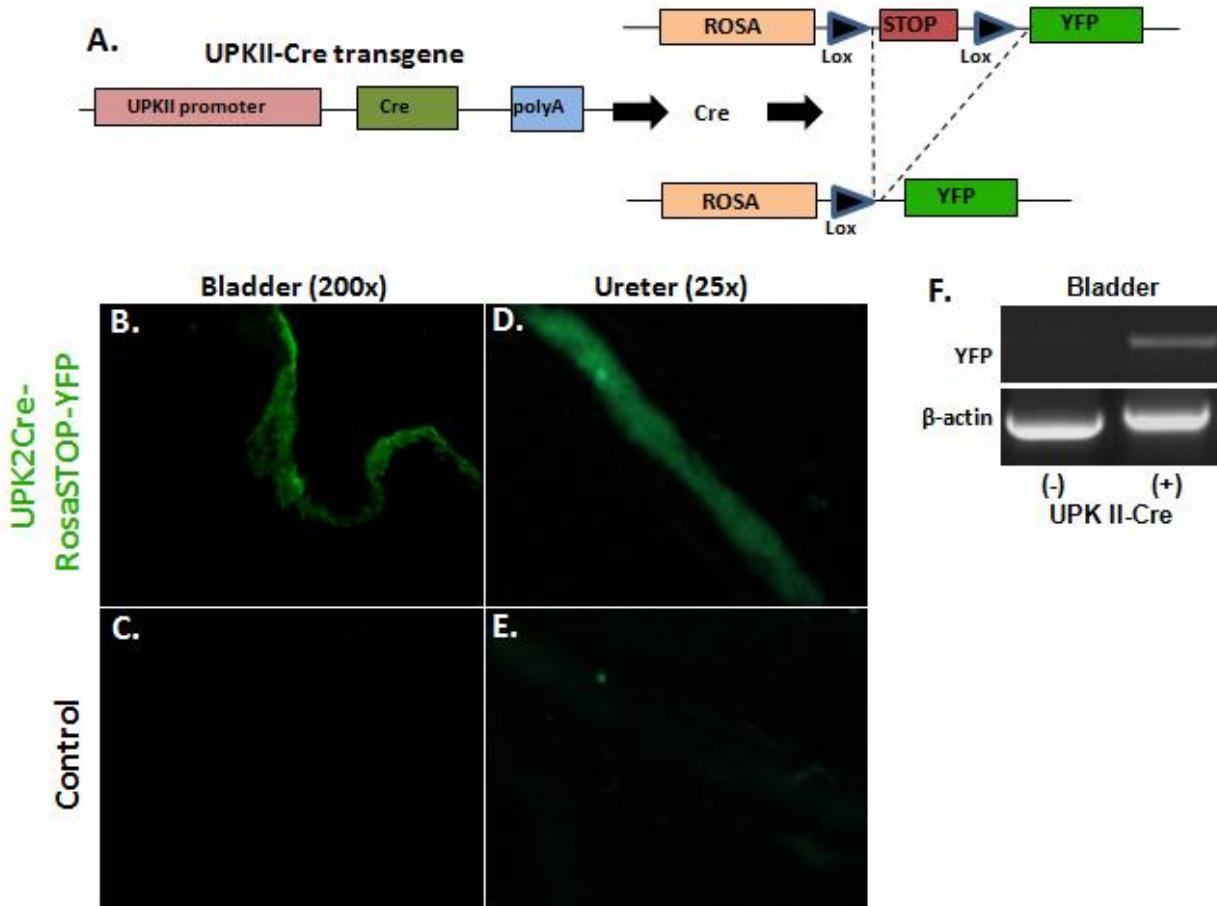


Figure Suppl. 2 . Reporter mouse UPK II-Cre;Rosa-STOP-YFP. A, DNA construct formed by the UPKII promoter and Cre-recombinase; the excision of the STOP cassette by Cre-recombinase generates urothelial-restricted expression of YFP. B-F, fluorescence was found only in bladder (B,C) and ureter (D,E) of UPKII-Cre;Rosa-STOP-YFP(+/+) reporter mice, but not in controls. F, PCR for recombinant UPKII-YFP was only positive in UPKII-Cre;Rosa-STOP-YFP(+/+) bladder.

Supplementary table 1. PCR primers used for cDNA synthesis and amplification of miRNA.

miRNA	Sequence	Stem-loop primer	Forward primer (5'→ 3')
RNU6B	CUGCGCAAGGAUGACACGCAAAU UCGUGAACGUUCCAUUUUU	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACAAAAATATGG	TGCGGCTGCGCAAGGATGA
miR-17	CAAAGUGCUUACAGUGCAGGUAG	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACCTACCTGC	TGCGGCAAAGTGCTTACAGT
miR-18a	UAAGGUGCAUCUAGUGCAGAUAG	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACCTATCTGC	TGCGGTAAAGGTGCATCTAGT
miR-19a	UGUGCAAUCUAUGCAAAACUGA	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACTCAGTTT	TGCGGTGTGCAAATCTATGC
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACCTACCTGC	TGCGGTAAAGTGCTTATAGT
miR-92a	UAUUGCACUUGUCCCGGCCUG	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACCAGGCCGG	TGCGGTATTGCACTTGTG
miR-21	UAGCUUAUCAGACUGAUGUUGA	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACTCAACATC	TGCGGTAGCTTATCAGACT
miR-205	UCCUCAUUCCACCGGAGUCUG	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACCAGACTCC	TGCGGTCTTCATTCCACC
miR-222	AGCUACAUCUGGUACUGGGU	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACACCCAGTA	TGCGGAGCTACATCTGGC
miR-129-5p	CUUUUUGCGGUCUGGGCUUGC	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACGCAAGCCC	TGCGGCTTTTGCAGGTCT
miR-34a	UGGCAGUGUCUUAGCUGGUUGU	GTCGTATCCAGTGCAGGGTC CGAGGTATTGCACTGGATA CGACACAAACAG	TGCGGTGGCAGTGTCTTAG
miR-145	GUCCAGUUUUCCCAGGAAUCCU	GTCGTATCCAGTGCAGGGTCCG AGGTATTGCACTGGATAACGAC AGGGATTC	TGCGGTGAGATGAAGCACT
Common reverse	-----	-----	Reverse primer (5'→ 3') GTGCAGGGTCCGAGGT

Supplementary Table 2. Primers for rt RT-PCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
VEGF-A	GGAGATCCTCGAGGAGCACTT	GGCGATTAGCAGCAGATATAAGAA
HIF-1 α	CAGCTTCCTCGGACACATAAG	CCACAGCAATGAAACCCTCCA
HIF-1 β	GAAATCTATCCCAGCATCAC	GACCACTATT CCTGAAATTCTC
TSP-1	ACTGACCGAGGAAGGGTCC	CCCGCTGTAGCTCTGTTCA
Dicer	GGTCCTTCTTGGACTGCCA	GCGATAACGTCTCCCTGA
Drosha	CACTCCA ACTACAAGAGCCA	ATTGCTTCTTCAA ACTCCGT

Supplementary Table 3. Level of expression of angiogenic markers by gender in UPKII-SV40 mice.*

	Females*	Males*	P**
VEGF	2.09	2.04	
TSP-1	0.60	0.47	0.59
HIF1a	1.17	0.74	0.12
HIF-1b	1.52	1.35	0.61
miR-107	--	--	--
miR-18a	7.86	4.08	0.47
miR-19a	5.54	3.18	0.36
miR-20	1.81	1.28	0.58
miR-17	3.01	1.93	0.41
miR-92	---	---	---
miR-34	1.10	0.73	0.315
miR-21	0.91	0.47	0.21
miR-205	0.57	0.38	0.25
miR-222	0.46	0.30	0.41
miR-145	0.21	0.21	0.95
miR-200c	1.05	0.77	0.49

* Levels of expression are related to the reference control group (normalized to 1).

** U-Mann Whitney test for comparison of means.