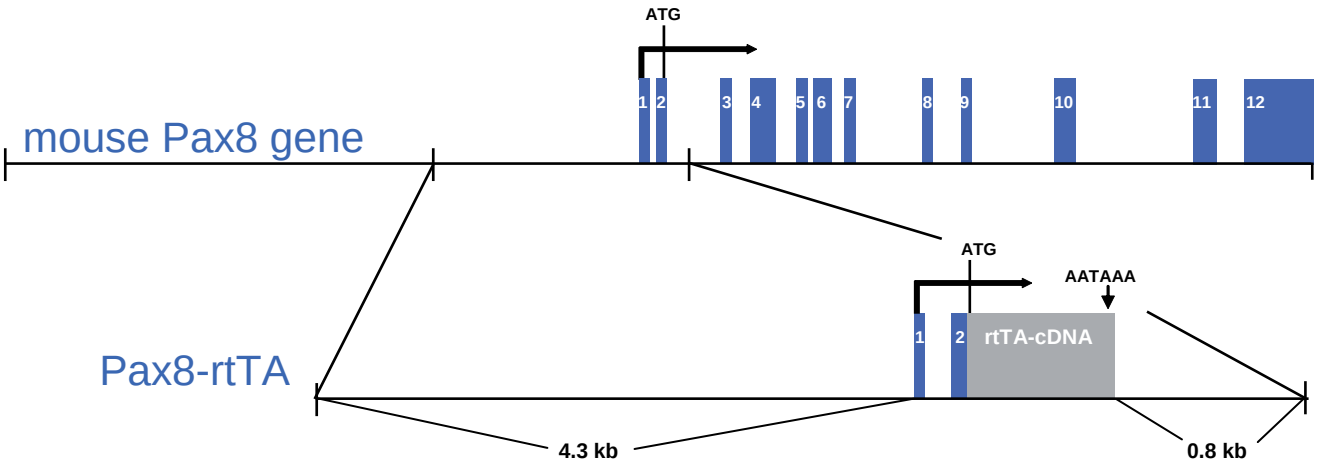
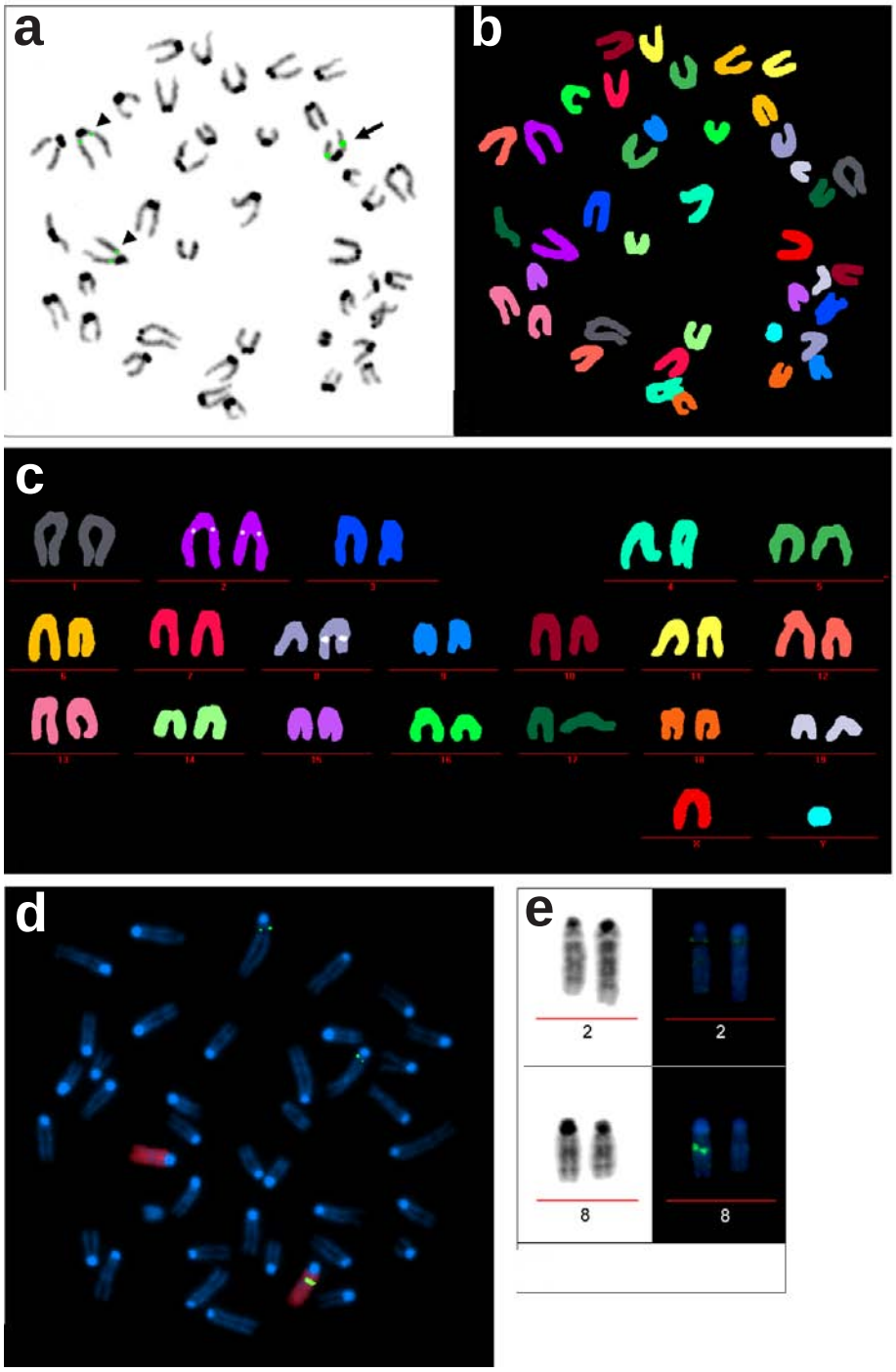


Supplementary Figure 1. Generation of the Pax8-rtTA transgenic line.
The exon-intron structure of the mouse Pax8 locus is shown above. Exons are indicated as numbered boxes. The recombinant rtTA-cDNA containing a consensus Kozak translational initiation site and a polyadenyation signal (AATAAA) was used to replace the endogenous Pax8 start codon (ATG) located within exon 2. The final Pax8-rtTA construct (shown below) contains 4.3 kb of upstream regulatory sequence, complete exon 1 and intron 1, part of exon 2 and 0.8kb of intron 2 of the murine Pax8 gene.



Supplementary Figure 2. Chromosomal mapping of the PAX8-rtTA probe by FISH.
FISH analysis using pPAX8-rtTA as probe and subsequent M-FISH were performed to determine the precise integration site of the PAX8-rtTA transgene in the mouse genome.
a Inverted DAPI staining of a metaphase spread after hybridization of the PAX8-rtTA probe.
b The same metaphase spread after the Re-FISH protocol and M-FISH using combinatorial labeled mouse chromosome painting probes showing the integration of Pax8-rtTA DNA on one copy of chromosome 8 (characterized by the dark gray classification color). Additionally, weak signals were seen on mouse chromosome 2 well corresponding to the endogenous Pax8 gene.
c Multicolor karyogram of the metaphase spread overlaid with the PAX8-rtTA signals in white color.
d Two color FISH analysis using the PAX8-rtTA probe (green) and chromosome 8-specific painting probe (red) confirms the integration site on chromosome 8.
e Inverted DAPI staining (left) and hybridization signals (right) allows mapping of the PAX8-rtTA probe to chromosome 8 band B2.

Supporting Online Material for

An Efficient and Versatile System for Acute and Chronic Modulation of Renal Tubular Function in Transgenic Mice

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SUPPLEMENTARY METHODS

Construction of Vector pPax8-rtTA. We used a pBluescript KS+ plasmid clone harbouring a 12kb EcoRI/EcoRI sub-genomic fragment of the mouse Pax8 gene as source DNA for the isolation of Pax8 promoter sequences[1]. A 5636 bp PshAI/EcoRI fragment thereof was derived and cloned into an EcoRV/EcoRI restricted pBluescript II KS vector. A 277 bp BamHI/SacII fragment encompassing the endogenous start ATG codon of the mouse Pax8 gene was replaced by a homologous 245 bp BamHI/SacII fragment generated by PCR using the 5' PCR primer 5'-AGTTTGGGGCTTCCTTGG-3' and the 3' primer 5'-AAACCGCGGGGCGCGCCAGTCGCTCACAGACTGAGG-3' adding novel AscI and SacII restriction sites. The resulting PCR reaction product was restricted with BamHI and SacII and used to replace the corresponding native BamHI/SacII fragment. The resulting vector was designated Pax8.

To generate the pPax8-rtTA construct, rtTA2^{s}-M2 encoding cDNA together with SV40 polyA sequences was amplified by PCR from the vector pUHrT 62-1[2]. PCR primers were rtTA-fwd1 5’- AAAGGCGCGCCAATACCACCATGTCTAGACTGG-3’ and rtTA-rev1 5’-AAACCGCGGATTTTACCACATTTGTAGAG-3’. After cutting the PCR-fragment with AscI/SacII, it was ligated into the AscI/SacII cloning site of the vector Pax8. The resulting plasmid was designated pPax8-rtTA.

As a result, the endogenous Pax8 start codon along with eight nucleotides residing immediately upstream and 29 bp located immediately downstream were replaced by an AscI cloning site followed by a consensus Kozak translational initiation site and the rtTA open reading frame.

Generation of Pax8-rtTA Transgenic Mice. To prepare DNA for microinjection, we restricted large-scale plasmid prep DNA (Qiagen Plasmid DNA Maxi Kit) with XhoI /NotI and purified the 6.6 kb insert released from 0.7% agarose gel using Concert Matrix Gel

Extraction Kit (Invitrogen). The linear DNA fragment was then microinjected into the pronucleus of one-cell fertilized mouse embryos obtained from superovulated F2 (C57Bl/6 x DBA) females. The injected embryos were surgically reimplanted into the oviducts of pseudopregnant recipient mice and allowed to develop to term.

PCR for genotyping.

Pax8-rtTA primers were:

ST1: 5'-CCATGTCTAGACTGGACAAGA-3'

ST2: 5'-CTCCAGGCCACATATGATTAG-3'

LC-1 primers were:

Cre3: 5′-TCGCTGCATTACCGGTCGATGC-3′

Cre4: 5′-CCATGAGTGAACGAACCTGGTCG-3′

NZL-2 primers were:

NZL-fwd1: 5′-TTACGATGCGCCCATCTACAC-3′

NZL-rev1: 5′-TTACCCGTAGGTAGTCACGCA-3′

Rosa26-R primers were:

Rosa26R-fwd1: 5′-AAAGTCGCTCTGAGTTTGTTAT-3′

Rosa26R-rev1: 5′-GCGAAGAGTTTGTCCTCAACC-3′

tet-o-TGFß1 primers were:

Tgfß-fwd2: 5′-CCCAGTGACTCACCGGAGTGG-3′

Tgfß-rev1: 5′-GTGTCTAGGCTCCAGATGTAGG-3′

tet-o-MYC primers were:

MYC-fwd1: 5'-GAGCCCCTGGTGCTCCATGAGG-3'

MYC-rev1: 5'-GCTGTGGCCTCCAGCAGAAGG-3'

Tsc1flox primers were:

Tsc1-fwd1: 5'-AGGAGGCCTCTTCTGCTACC-3'

Tsc1-rev1: 5'-CAGCTCCGACCATGAAGTG-3'

The PCR conditions were set as follows:

1 cycle of 3min 94°C, 35 cycles of (30s 94°C, 30s 52-60°C, 30s 72°C), 1 cycle of 7 min 72°C.

Fluorescence in situ hybridization (FISH). We hybridized metaphase spreads from fibroblasts cells derived from Pax8-rtTA transgenic mice with the PAX8-rtTA probe and took images using a Leica DM RXA RF8 epifluorescence microscope (Leica Mikrosysteme GmbH, Bensheim, Germany) equipped with a Sensys CCD camera (Photometrics, Tucson, AZ). Subsequently, the Re-FISH protocol was performed[3] and metaphase chromosomes were hybridized with mouse chromosome painting probes according to the multicolor FISH (M-FISH) protocol[4]. For evaluation, the same metaphase spreads were acquired using highly specific filter sets (Chroma Technology, Brattleboro, VT). The images were processed on the basis of the Leica MCK software (Leica Microsystems Imaging Solutions, Cambridge, United Kingdom) and presented as multicolor karyograms. Additionally, we performed a two-color FISH experiment using the PAX8-rtTA probe along with a probe specific for mouse chromosome 8 to confirm these results.

DIPS-PCR. We carried out DIPS-PCR according to Luft et al. (2001)[5]. Briefly, Pax8rtTA positive tail genomic DNA (0.6 μg) was restricted with Sau3AI (10 units) in a volume of 20 μl at 37°C overnight, followed by heat inactivation of the enzyme, according to the manufacturer's instructions (New England Biolabs, Beverly, MA). Ligation of Sau3AI-specific adapters (50 pmol) to the restricted genomic DNA was performed by adding 5 U of T4-DNA ligase (Roche Mannheim, Germany), ATP and DTT (final concentration of 2 mM

each) in a total volume of 24 µl at 14°C overnight. The ligation reaction was diluted to 40 µl with sterile water. Then 2 µl of the ligation products were subjected to two rounds of PCR amplification using nested Pax8rtTA-specific primers (Pax8-rev1 5'-GAATGGAGGTGTGGGAAGAG-3' and Pax8-rev2 5'-CTATCAATCTTAGTGGGTGATG-3') in combination with the adapter-specific primer AP1. Resulting PCR products were purified, cloned into bacterial plasmids and sequenced.

Determination of serum doxycycline by luciferase assay. We determined serum Dox concentrations by measuring the activity of a tetracycline-dependent luciferase reporter gene in HeLa cells, using Dox standards diluted in mouse sera as a reference. In brief, mouse sera from Dox-treated mice were added to the HeLa culture medium and luciferase activity was assayed 16 hours later. Luciferase induction was linear up to 50ng /mL doxycycline, with a detection limit of 5ng/mL. We performed all assays at least in duplicate measurements in three independent experiments.

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