SUPPLEMEMTARY MATERIAL TO: "Cubilin is essential for albumin reabsorption in the renal proximal tubule" by S. Amsellem et al.

SUPPLEMENTARY METHODS

Generation of mice. A 12-kb genomic clone encompassing exons 11 to 15 of Cubn was isolated from a 129/X1SvJ genomic library and subcloned in NotI/EcoRV sites of pBluescript. Using standard cloning techniques, an EcoRI-*loxP* site and a *loxP-Hygro-loxP* cassette were inserted in introns 13 and 14 respectively. This construction was cut with NotI and EcoRV to release the vector and the linearized insert was electroporated in embryonic stem (ES) cells following standard methods. ES cell clones were tested by long-range PCR using external primers and Southern blotting using external 5' probe as indicated in Figure S1. One screen positive clone was expanded, karyotyped and injected in C57BL/6 blastocysts. Germ line transmission was obtained for several chimeric mice. Homozygous $Cubn^{lox/lox}$ mice had normal histology, normal renal function and did not display any increased protein or albumin excretion.

Genotyping. For each mating, genotyping PCRs were carried out to identitfy the offsprings bearing the desired alleles. Multiplex PCRs (primers in Table 1, Figure S2) were performed using the following protocol: denaturation at 94°C x 3 min, followed by 35 cycles of denaturation at 94°C x 30 sec, annealing at 60°C for 30 sec ending with elongation at 72°C for 45 sec and a final elongation step at 72°C for 7 min. Mouse breeding and handling were carried out in a certified animal facility according to procedures that were approved by the local animal care and experimentation authorities.

RT-PCR. Small samples of renal tissue were obtained surgically and immediately frozen in liquid nitrogen. RNA preparation and reverse transcription was carried out using kits from

Macherey-Nagel (Hoerdt, France) and Invitrogen (Cergy-Pontoise, France) respectively following manufacturers' instructions. PCR protocol was as described above. Primers "rtf" and "rtr" (Table 1) were selected on exon 13 and 15, i.e. upstream and downstream of the targeted exon 14.

SUPPLEMENTARY FIGURES AND TABLE

Supplementary figure S1. Generation of a floxed Cubn exon 14 allele.

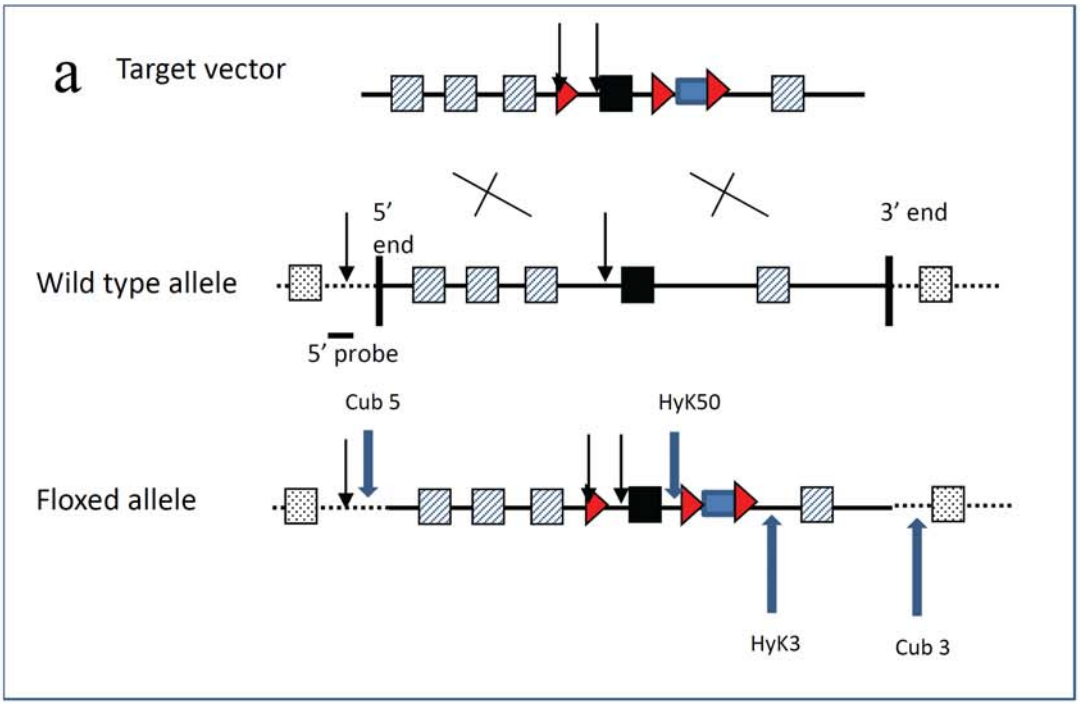
a: A 10.5 kb targeting vector, consisting of a *loxP* site (in red) in intron 13 and a *loxP-Hygro-loxP* cassette in intron 14, was constructed, linearized and electroporated into ES cells. b: ES cell clones were screened by Southern blot hybridization using a 5' external probe. c: long range PCR using external primers. Position of the primers used for long range PCRs are shown in a (bold arrows) as well as the position of the 5' probe and EcoRI sites (thin arrows) used for the Southern blot. d: RTPCRcarried out on renal cortex extracts confirms deletion of exon 14.

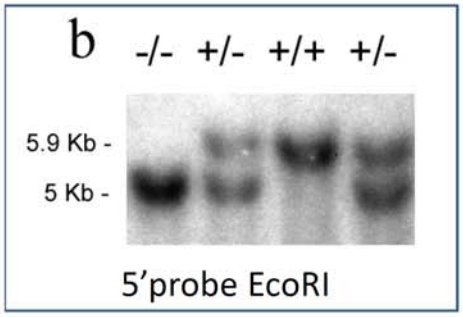
Supplementary figure 2. Genotyping of Cubn alleles.

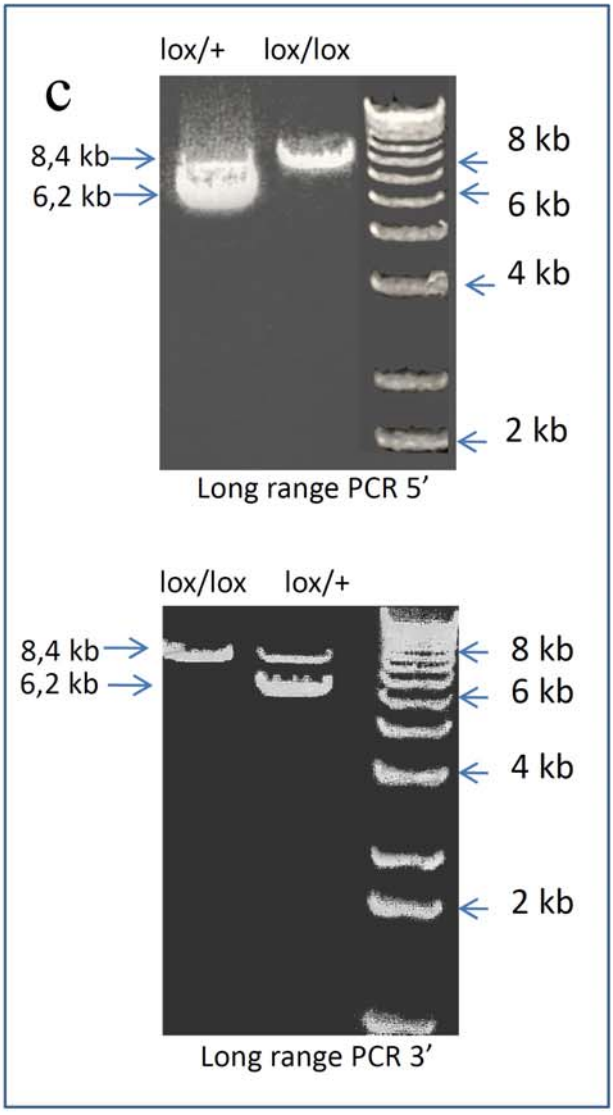
a: Scheme indicates positions of primers on floxed and recombined allele; b: Representative PCR results using primers lox5, hyk3, hyk51 to identify wild type or recombined null alleles or primers lox5, lox3 to identify floxed and wild type alleles. Note that the PCR parameters selected preclude synthesis of fragments>1.5 kb.

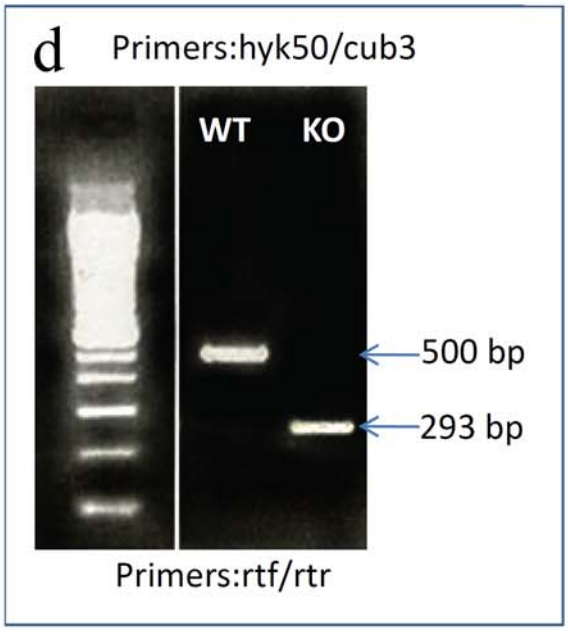
Table 1. Primers used

cub 3	5' CTTCAACTTCGGAGACCTGCA 3'
hyK50	5' CCATCCACCTCTCATTTGCT 3'
cub 5	5' CCTTCCTGACGAAGAAGTGC 3'
hyk3	5' AAGGGACTAGGGACAGGTACATC 3'
hyk51	5’ ATGCTCAGCTGGGACTGTCT 3’
lox 5	5' GTTGTCAGAACCGATGAGGA 3'
lox3	5'TCAGGGCCTAGAGCTAGACA 3'
rtf	5’ GGATCCTCTCAGGGACACAA 3’
rtr	5’ CTCTCCAGGCTCAAGGTTCC 3’
CreF	5’ GGACATGTTCAGGGATCGCCAGGCG 3’
CreR	5’ GCATAACCAGTGAAACAGCATTGCTG 3’

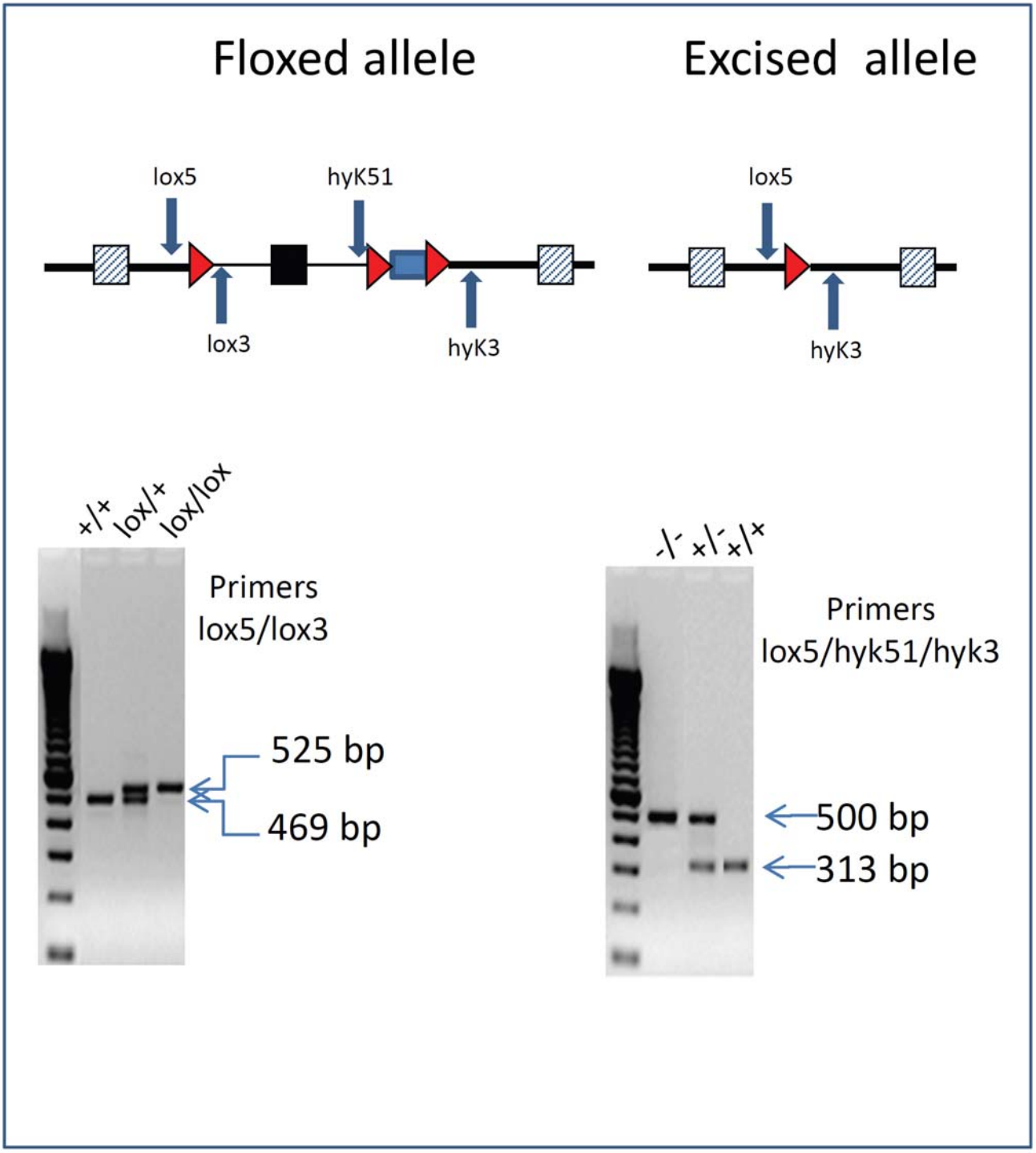








Suppl. Figure 1



Suppl. Figure 2