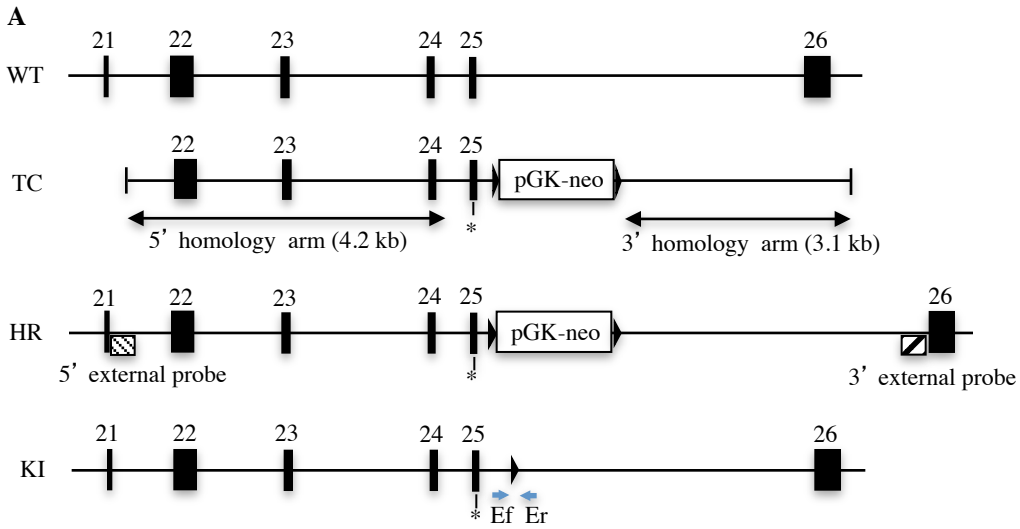
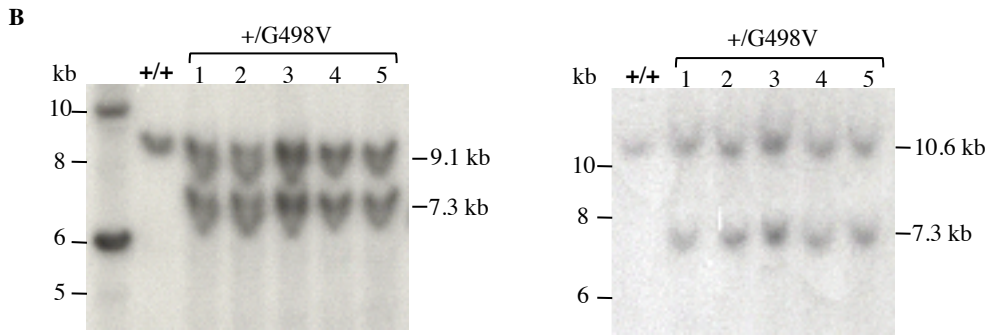
Supplemental Material

Figure 1: Generation of *Col4a1 G498V* HANAC mutant mice

(A) Targeting strategy. WT : wild-type (WT) allele; TC : targeting construct containing the 5' homology arm (4.2kb), the central fragment with the Gly498Val (G498V) mutation (*), the selection cassette pGK-neo flanked by two loxP sites (black arrows) and the 3'homology arm (3.1kb) ; HR : homologous recombinant allele obtained after homologous recombination in embryonic stem (ES) cells; KI : targeted floxed-out knock-in allele. (B) Southern blot using genomic DNA of *Col4a1*$^{+/+}$and *Col4a1*$^{+/G498V}$ ES cells. Left panel : digestion with ScaI/BglI and hybridization with the 5' external probe (left hatched square under HR allele in A), showing a 9.1kb band for wild-type allele, and a 7.3kb band for the G498V mutant allele. Right panel : digestion with DrdI and hybridization with the 3' external probe (right hatched rectangles under HR allele in A), showing a 10.6kb band for wild-type allele, and a 7.3kb band for the G498V mutant allele. (C) Validation of the pGly498Val point mutation by tail DNA sequencing of wild-type (*Col4a1*$^{+/+}$) and heterozygous (*Col4a1*$^{+/G498V}$) mutant mice. (D) PCR genotyping using Ef/Er primers (indicated in A) of tail DNA from wild-type (+/+), heterozygous (+/*G498V*) and homozygous (*G498V/G498V*) mice. The wild type allele corresponds to the lower 237bp band and the mutant allele to the upper 324bp band.





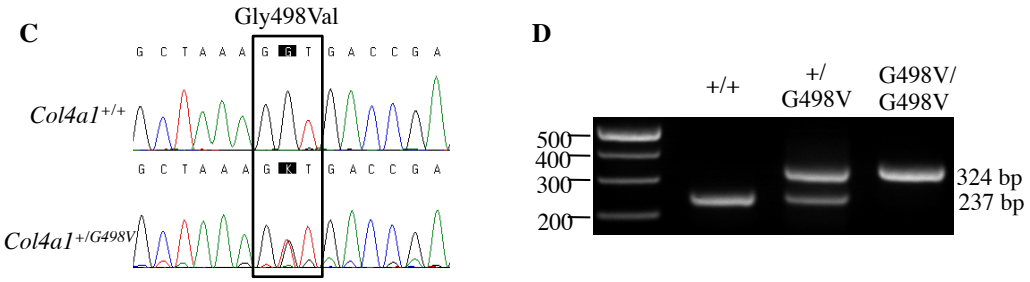


Figure 2: Post-natal evolution of kidney abnormalities in *Col4a1*^{G498V} newborn mice.
(A) Quantification of urine albumin in newborn *Col4a1*^{G498V} mutant mice at day 0 (P0) and day 7 after birth (P7). Values represent mean ± SEM. (B) Spot urine analysis by SDS-PAGE in homozygous (G498V/G498V) mice at postnatal day 0, day 2 (P2), day 4 (P4) and P7 showing progressive decrease of urine albumin excretion. (C) Kidney sections of *Col4a1* homozygous mutant mice at P0 and P7, showing the recovery of the glomerular and tubular lesions in homozygous renal tissue at P7. Note the persistence of tubular erythrocyte casts at P7 (arrows). Periodic Acid Schiff, Scale bar: 250μm. (D) Glomerular density in 6-month male kidneys. Glomeruli were counted in cortical areas of one-millimeter depth from the renal capsule which covered the whole longitudinal kidney sections (left panel, yellow circles identified glomeruli) (+/+, n=4; +/G498V, n=4; G498V/G498V, n=4).

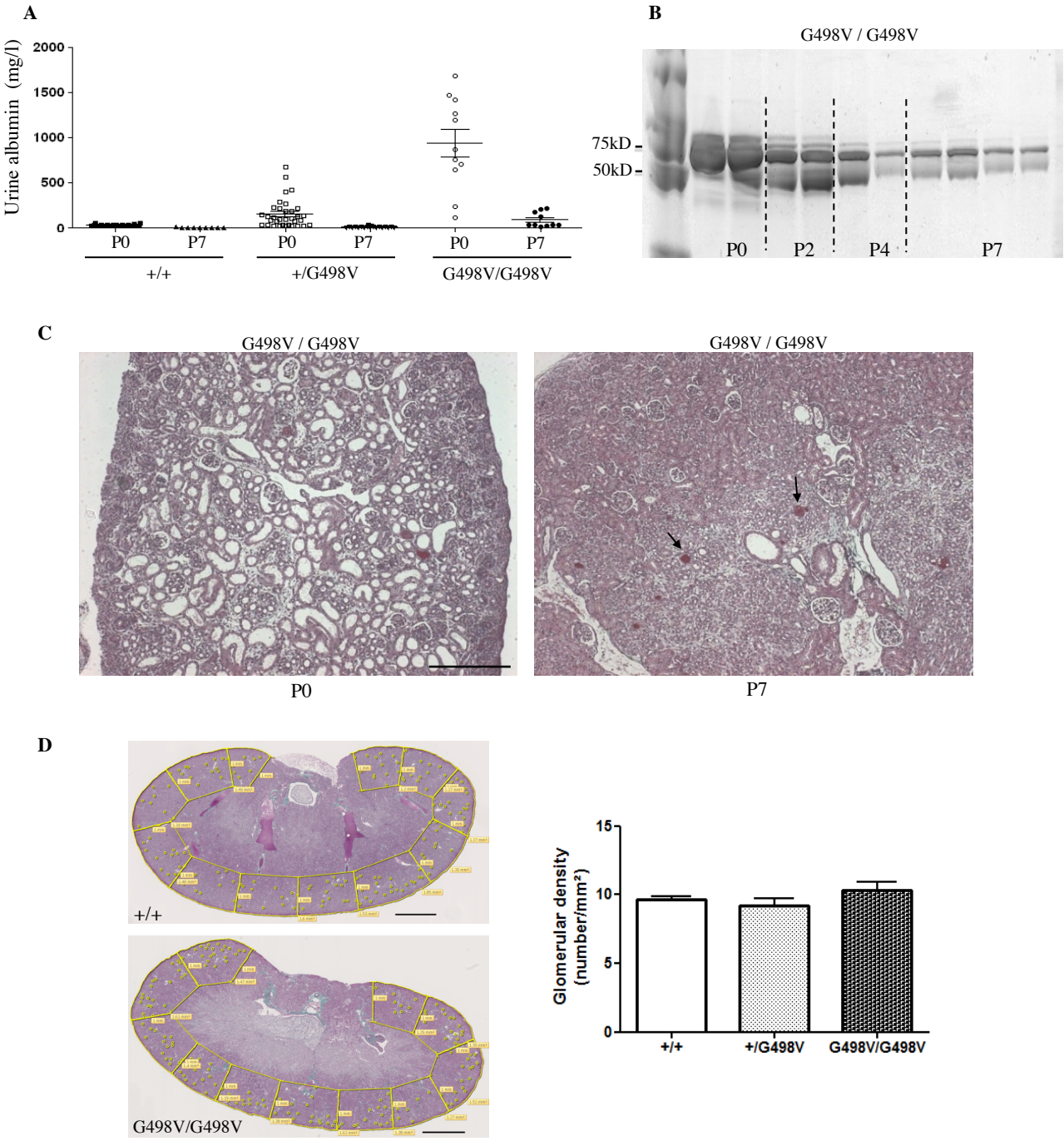


Figure 3: Renal phenotype of adult *Col4a1*^{G498V} mutant mice. (A) Kidney sections from 1-month animals. At low magnification, renal tissue of mutant heterozygous (+/G498V) and homozygous (G498V/G498V) animals did not show significant tubular or glomerular alteration (Masson's Trichrome). Scale bar: 200μm. On higher magnification, parietal epithelial cells (PECs) of a subset of mutant glomeruli showed a cuboid shape with enlarged nuclei (arrow), contrasting with the flat aspect of normal PECs. Periodic acid Schiff. Scale bar: 20μm. (B) Immunohistochemical staining of 12-month adult kidney sections with anti-CD3 and F4/80 antibodies. In mutant kidneys of both genotypes, periglomerular and perivascular inflammatory infiltrates were mostly composed by T-lymphocytes surrounded by macrophages (G: glomerulus, A: arteriole). Scale bars: 50μm. (C) High magnification showing a renal arteriole with normal structure surrounded by a dense inflammatory infiltrate in a 12-month homozygous mice. Masson's Trichrome. Scale bar: 20mm^2 (D) Ultrastructural analysis of the Bowman's capsule and PECs in 6-month adult kidneys. In wild-type mice, PECs had the characteristic flattened appearance with small body size and flat small nuclei whereas in homozygous mutants, they were enlarged with a cuboid shape and enlarged nuclei. White arrows indicate irregular and thickened Bowman's capsule. Scale bar: 2μm.

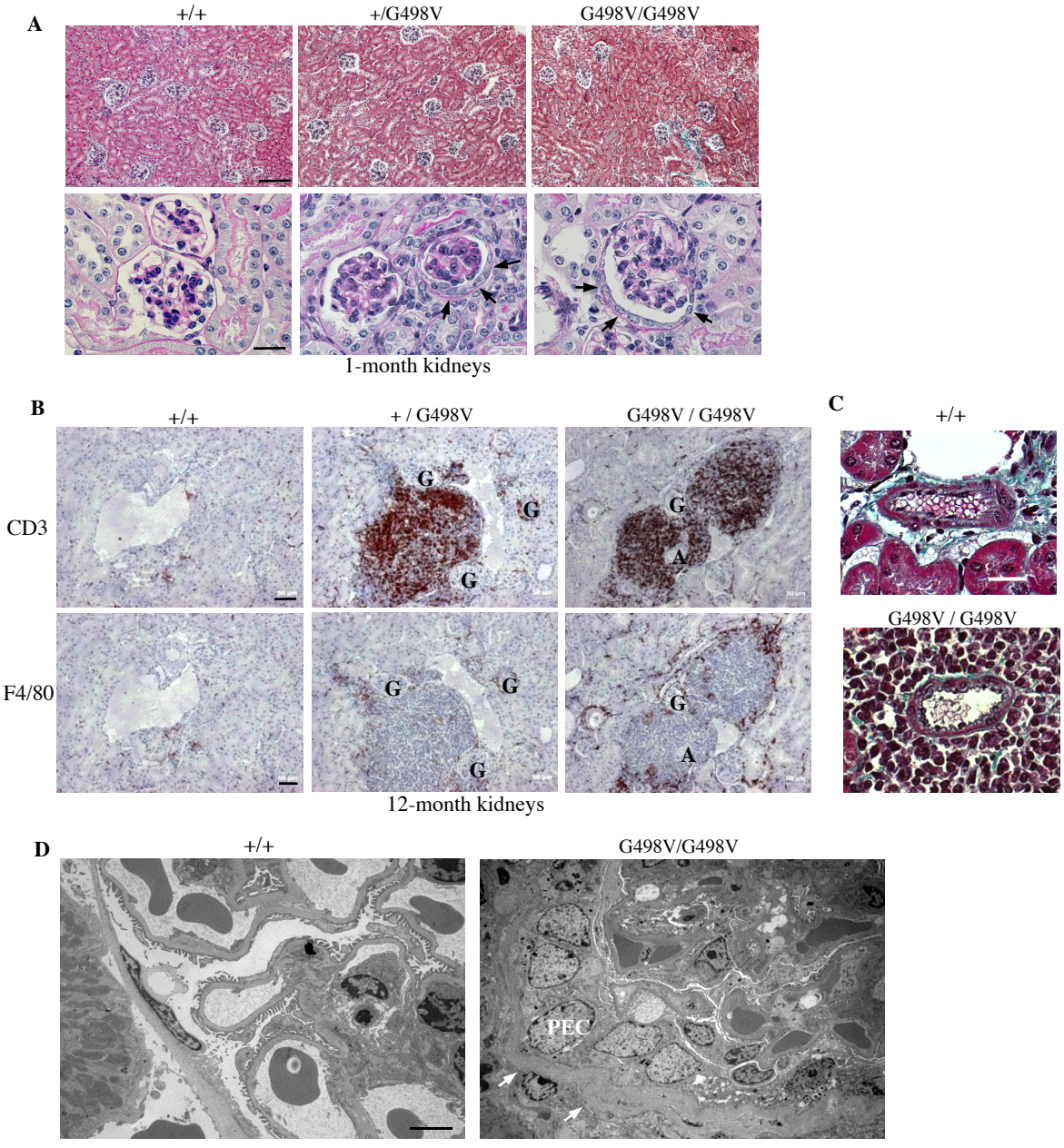
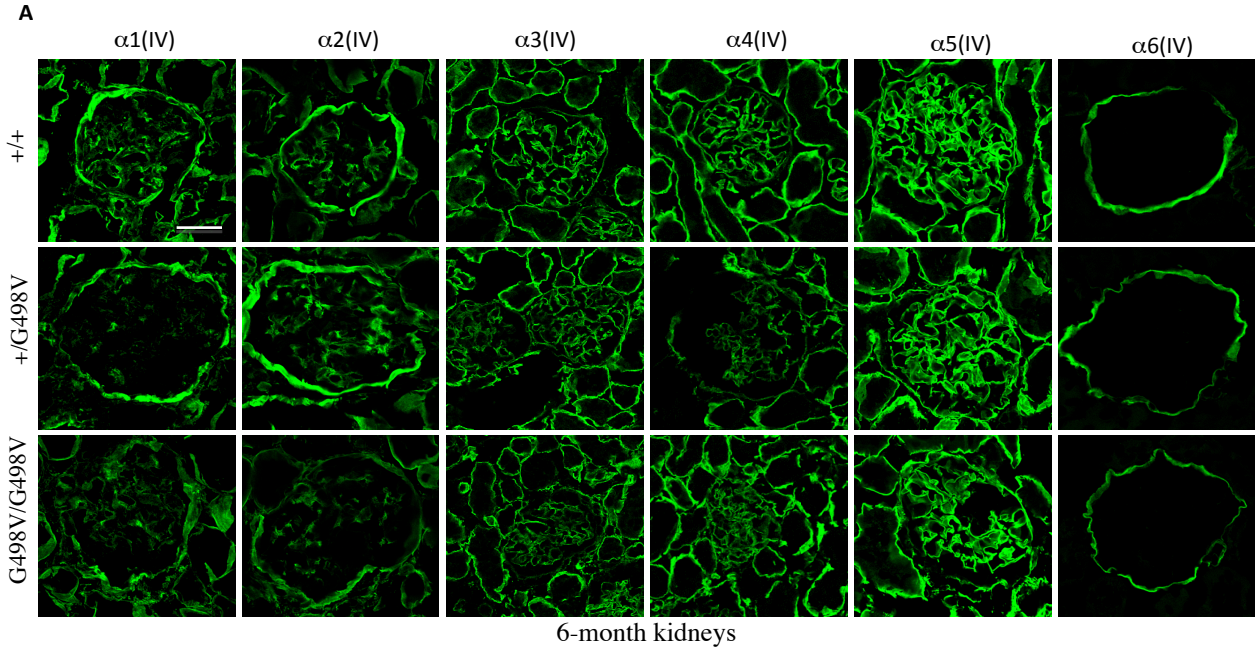


Figure 4: Expression of a chains of type IV collagen and extracellular matrix proteins. (A) Immunofluorescence with antibodies specific for the α1-α6(IV) chains of collagen IV in kidney sections of 6-month old wild-type (+/+) and *Col4a1* G498V mutant mice. Note decreased expression of α1 and α2 mostly in the Bowman's capsule and unchanged expression of the other chains in homozygous mutants. Scale bars: 25μm. (B) Expression of laminin α2 and nidogen, fibronectin and collagen I in 6-month old wild-type and *Col4a1* G498V mutant kidneys was unchanged. Scale bars: 25 μm.



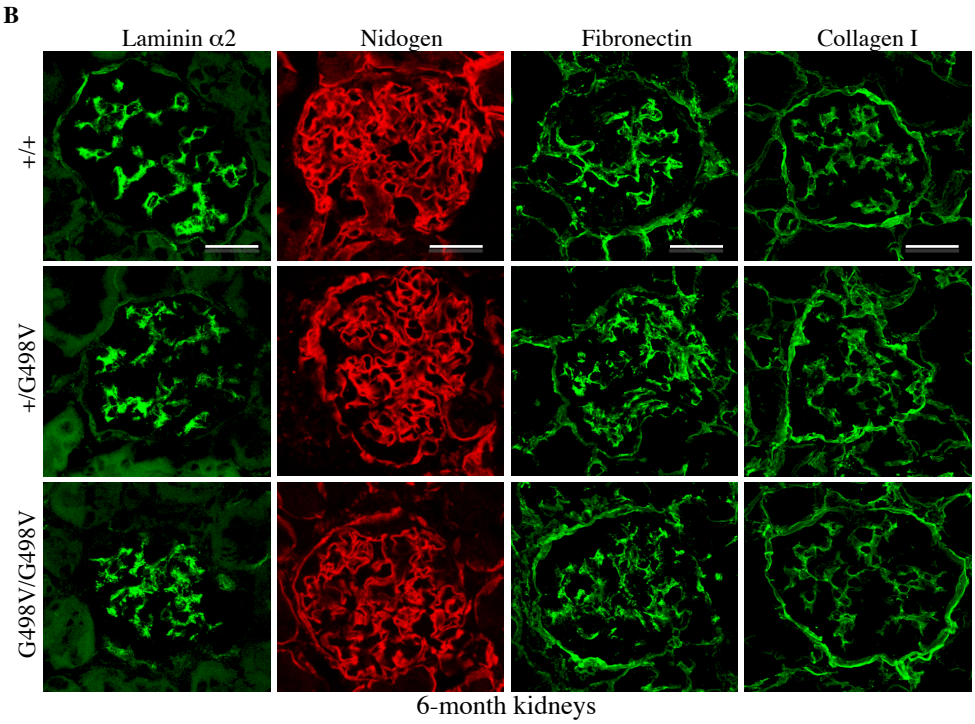


Figure 5: Mean body weight and kidney weight of *Col4a1*^{G498V} males and females at 6 and 12 months of age.

Values represent mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001, compared to wild-type animals (+/+); $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$ p<0.001 compared to heterozygous animals (+/G498V).

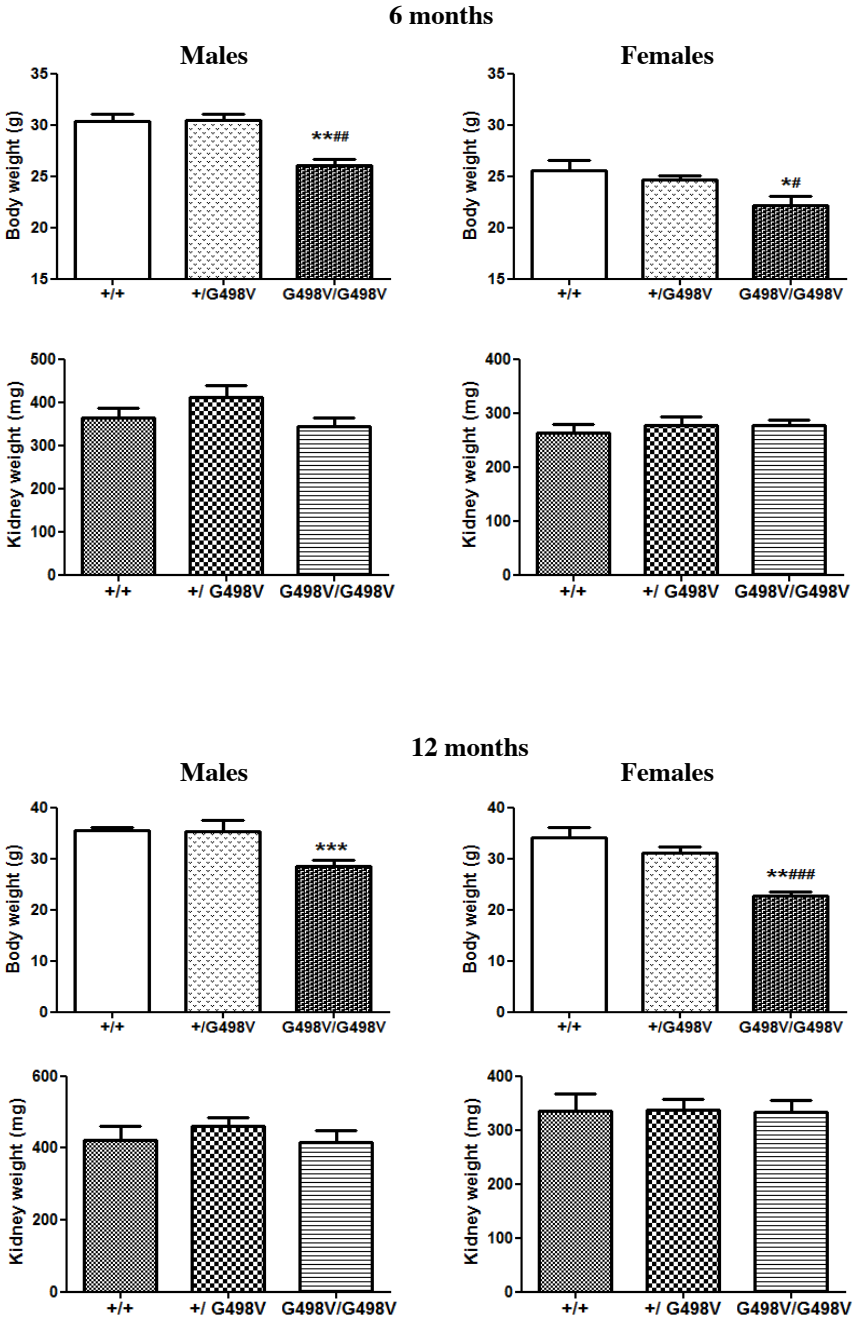


Figure 6: Phenotypic alterations of glomerular parietal epithelial cells (PECs) in one-month *Col4a1*^{G498V} mutant mice. (A) Immunohistochemical staining showed overexpression of claudin-1 and induction of CD44 in parietal epithelial cells (PECs) of 1-month-old heterozygous (+/G498) and homozygous (G498V/G498V) *Col4a1* mutant kidneys.
(B) Expression of DDR1, ILK, paxillin and β-catenin was strongly induced in PECs of 1-month-old *Col4a1*^{G498V} mutant mice. Scale bar: 100μm.

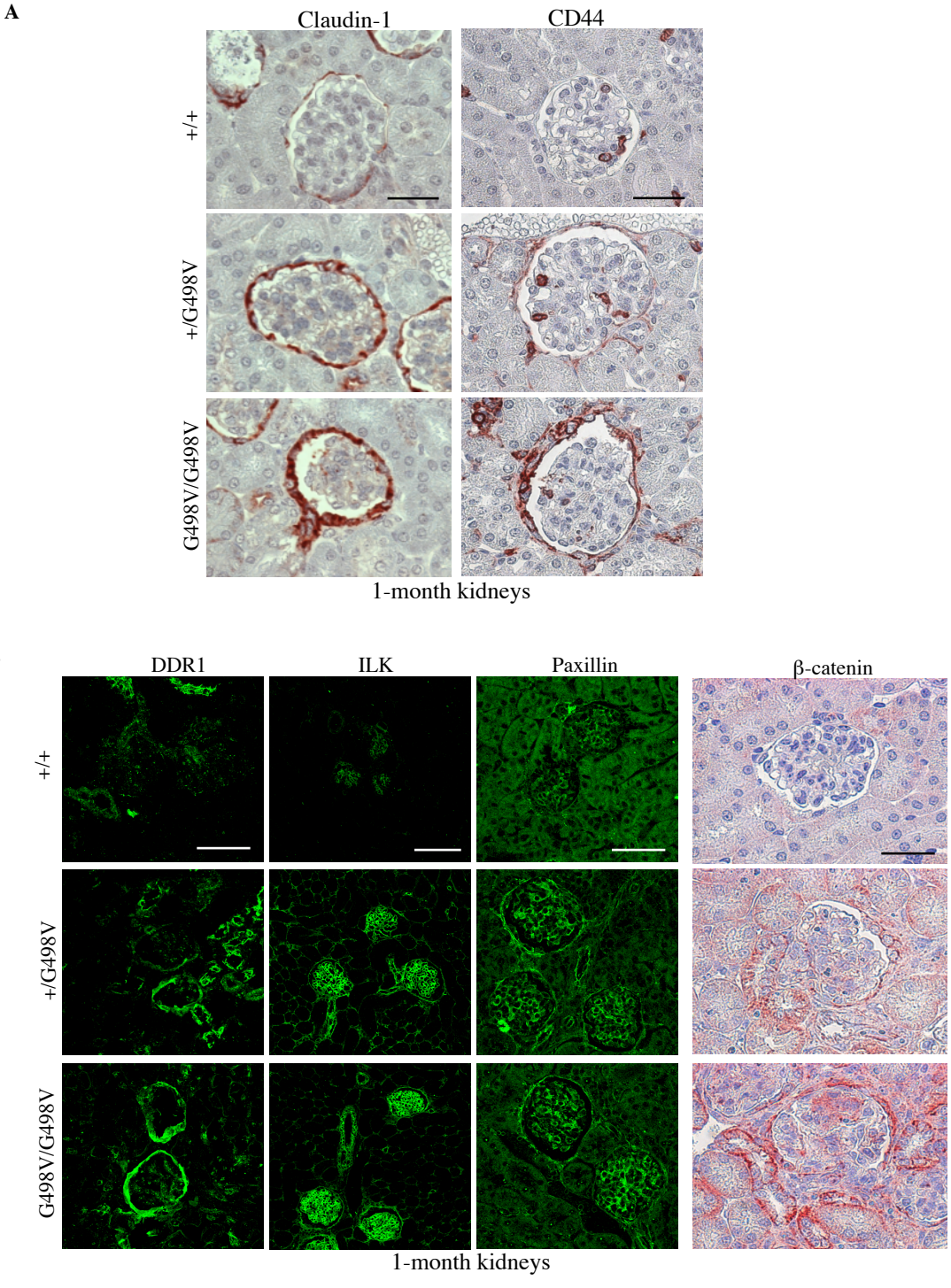


Table 1: Antibodies used for immunofluorescence and immunochemistry studies

a-SMA	rabbit	1/1000	ab5694	Abcam
b-catenin	rabbit	1/1000	sc-7199	Santa Cruz
CD3	rabbit	1/400	A0452	Darko
CD44	Rat	1/100	550538	BD Pharmingen
Claudin 1	rabbit	1/400	ab15098	Abcam
Collagen I α1α2	rabbit	1/500	600-401-103-01	Rockland
Collagen IV α1α2	rabbit	1/500	600-401-106-05	Rockland
Collagen IV α1	rat	1/50	H11	Y. Sado, Japon
Collagen IV α2	rat	1/50	H22	
Collagen IV α3	rat	1/50	H31	
Collagen IV α4	rat	1/50	RH42	
Collagen IV α5	rat	1/50	H53	
Collagen IV α6	rat	1/50	B66	
DDR1 (C-20)	rabbit	1/100	sc-532	Santa cruz
F4/80	rat	1/800	MCA497G	AbD serote
Fibronectin	rabbit	1/400	ab2413	Abcam
ILK (EPR1592)	rabbit	1/200	04-1149	Millipore
Laminin a2 (4H8-2)	rat	1/50	sc-59854	Santa Cruz
Nephrin	guinea pig	1/200	GP-N2	PROGEN
Nidogen	rat	1/50	MAB1946	Millipore
Paxillin (C-18)	goat	1/200	sc-7336	Santa Cruz
Perlecan	rat	1/100	05-209	Millipore
Podocin	rabbit	1/1000		Gift from Pr Antignac

Table 2: Primers and PCR condition used for quantitative RT-PCR.

Housekeeping genes :
B2M : β2-microglobuline
Fw : CCTGTATGCTATCCAGAAAACCCCT
Rev: CGTAGCAGTTCAGTATGTTCGGCTT
Gusb : Beta-D-glucuronidase
Fw : CTGCGGTTGTGATGTGGTCTGT
Rev: TGTGGGTGATCAGCGTCTTAAAGT
Hprt1 : Hypoxanthine phosphoribosyl -transferase 1
Fw : GTTGGATACAGGCCAGACTTTGTT
Rev: AAACGTGATTCAAATCCCTGAAGTA
Ppia : Peptidylprolyl isomerase A
Fw : CCAAACACAAACGGTTCCCAGT
Rev: GCTTGCCATCCAGCCATTCA
Tbp : TATA box binding protein
Fw : TGACCTAAAGACCATTGCACTTCGT
Rev: CTGCAGCAAATCGCTTGGGA

Target genes :
MMP2 : Matrix metalloproteinase 2
Fw : TTCCCTAAGCTCATCGCAGACTC
Rev: TTGAAGAAGTAGCTATGACCACCAC
MMP9 : Matrix metalloproteinase 9
Fw : TCTTTGAGTCCGGCAGACAATCC
Rev: GAACTTCCAGTACCAACCGTCC
MMP14 (MT1-MMP) : Membrane type 1 matrix metalloproteinase
Fw : CCTCAACCCAGAACTACCTCTC
Rev: GAACCATCGCTCCTTGAAGAC
TIMP1 : Tissue inhibitor of metalloproteinase 1
Fw : AATCAACGAGACCACCTTATACCA
Rev: ATTTCCCACAGCCTTGAATCC
TIMP2 : Tissue inhibitor of metalloproteinase 2
Fw : GACACGCTTAGCATCACCCAG
Rev: ACCCAGTCCATCCAGAGGCA

PCR condition :
95°C for 5 min, and 45 cycles at 95°C for 20 s and 65°C for 15 s, then 72°C for 15 s.