**Supplemental Figure 1. Experimental design of the study**

(A) Experimental timeline for the determination of sodium balance in metabolic cages and amiloride-sensitive natriuresis before and after induction of nephrotic syndrome in the same mice. Doxycycline induction was done over 14 days and end of induction was designated as day 0.

(B, C) Experimental timeline for the study of sodium retention during the course of experimental nephrotic syndrome and for the study of the effect of aprotinin on sodium retention. These experiments were done in different mice.



**Suppl. Fig 2. Normal kidney function and absence of kidney damage in *nphs2Δipod\*plg-/-* mice**

1. Growth curve of uninduced *nphs2Δipod\*plg+/+* and *nphs2Δipod\*plg-/-* mice before start of induction treatment at day -14.

(B-E) Normal kidney histology, function, and absence of albuminuria in uninduced *nphs2Δipod\*plg+/+* and *nphs2Δipod\*plg-/-* mice.

(F-G) Sodium handling of uninduced *nphs2Δipod\*plg+/+* and *nphs2Δipod\*plg-/-* mice showing adequate response to a low salt diet.

Arithmetic means ± SEM, # indicates significant difference to baseline value, \*indicates significant difference between genotypes. U: uninduced, U+L: uninduced + low salt diet.



**Supplemental Figure 3. Food (A) and fluid intake (B) as well as body weight (C), body weight gain (D) and the area under the body weight curve (E) during the induction (day -14 to 0) and the nephrotic phase (day 0 and later) in *nphs2Δipod\*plg+/+* and *nphs2Δipod\*plg-/-* mice.**

**(F, G) Chronological evolution of proteinuria, urinary amidolytic activity, urinary sodium excretion and body weight in *nphs2Δipod\*plg+/+* (F) and *nphs2Δipod\*plg-/-* mice (G).**

Arithmetic means ± SEM, # indicates significant difference to baseline value.

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**Supplemental Figure 4. Specificity of the bands obtained with antibodies against α- and γ-ENaC subunits in kidney cortex from uninduced *nphs2Δipod\*plg+/+* mice**

(A, B) Localisation of the immunogenic sequences of the used antibodies against the murine α- and γ-ENaC subunit in relation to the cleavage sites. The antibody against α-ENaC is upposed to detect full-length α-ENaC and a N-terminal fragment with a mass of 26 kDa. The antibody against γ-ENaC is supposed to detect full-length γ-ENaC and a C-terminal fragment with a mass of 72 kDa after furin cleavage and another band at 65 kDa representing fully cleaved γ-ENaC.

(C) Administration of the blocking peptide for α-ENaC attenuated the bands at 26 and 87 kDa.

(D) Administration of the blocking peptide for γ-ENaC (Stressmarq) attenuated bands at 84 and 72 kDa while the band at 57 kDa was only partially blocked.



**Supplemental Table 1. Primers used for genotyping.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **gene** | **sense/forward**  **5’🡪3’ orientation** | **antisense/reverse**  **5’🡪3’ orientation** | **amplicon** | **reference** |
| Nphs2tm3.1Antc | CCAGCATCCCATTAGATAGATGAGG | GCATCCAAATGATCAGAGTTCCCAGG | 236 / 286 bp (wild-type / floxed allele) | 1 |
| Tg(Nphs1-rtTA\*3G)8Jhm | GAAGCAGCAGAATGAGTTCACACTGGGTCC | ACTTTGCTCTTGTCCAGTCTAGACATGGTG | 400 | 2 |
| Tg(tetO-cre) 1Jaw | GCATAACCAGTGAAACAGCATTGCTG | GGACATGTTCAGGGATCGCCAGGCG | 280 | 3 |
| Bl6-Plg+/+ and Plgtm1Jld | TCAGCAGGGCAATGTCACGG (wt) and GCACAGCTGCGCAAGGAACGCC (ko) | CTCTCTGTCTGCCTTCCATGG (wt) and AAGATGGATTGCACGCAGGTTCTC (ko) | 450 / 230 (wild-type / ko allele) | 4 |

**Supplemental Table 2. PCR conditions for genotyping.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **gene** | **denaturation** | **annealing** | **extension** | **cycles** |
| Nphs2tm3.1Antc | 94 ºC, 30 seconds | 62 ºC, 45 seconds | 72 ºC, 30 seconds | 40 |
| Tg(Nphs1-rtTA\*3G)8Jhm | 94 ºC, 30 seconds | 57 ºC, 30 seconds | 72 ºC, 45 seconds | 40 |
| Tg(tetO-cre)1Jaw | 94 ºC, 30 seconds | 57 ºC, 30 seconds | 72 ºC, 60 seconds | 35 |
| Bl6-Plgtm1Jld | 94 ºC, 30 seconds | 60 ºC, 60 seconds | 72 ºC, 60 seconds | 30 |

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