

## **APPENDIX**

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## Appendix Figure legends

### Appendix Figure S1.

A. Magnetic resonance renal volume to body weight ratio from control and *Lkb1*<sup>ΔTub</sup> mice at 16 weeks.

B. Periodic acid–Schiff (PAS) staining of *Lkb1*<sup>fl/+</sup>.*KspCre* mice at 23 weeks. Representative image of *n*=8 mice. Scale bar: 1mm.

C, D. Urinary flow rate (B) and urine osmolality (C) from control and *Lkb1*<sup>fl/+</sup>.*KspCre* mice at 23 weeks.

E. Kidney weight (KW) to body weight (BW) ratio from control and *Lkb1*<sup>fl/+</sup>.*KspCre* mice at 23 weeks.

Data information: In (A, C - E), each circle represents one individual mouse. Bars indicate mean. Mann–Whitney, *ns* not statistically different. In (C - E), values from littermates controls are the same as shown in Figure EV1H, I, K at 23 weeks.

### Appendix Figure S2.

A, B. Western blot (A) and quantification (B) of AMPK and mTORC1 signalling pathway in kidney medulla lysates from control and *Lkb1*<sup>ΔTub</sup> mice at 5 weeks.

C, D. Western blot (C) and quantification (D) of AMPK and mTORC1 signalling pathway in kidney medulla lysates from control and *Lkb1*<sup>ΔTub</sup> mice at 14 weeks.

E. Staining of pAMPK $\alpha$  and thick ascending limb of Henle (Tamm-Horsfall expressing, THP) in kidneys from 5 week old control and *Lkb1*<sup>ΔTub</sup> animals. Representative images of 7 mice/group. pAMPK $\alpha$  is present at the apical surface of THP positive tubules in both control and *Lkb1*<sup>ΔTub</sup> mice, where *KspCre* is active. Scale bar: 50 $\mu$ m.

F. pS6RP immunostaining in kidneys from control and *Lkb1*<sup>ΔTub</sup> mice at 5 and 23 weeks. Representative images of *n*=5 mice/group. Scale bars: 50μm.

G. Quantification of pS6RP positive tubular cells in kidneys from control and *Lkb1*<sup>ΔTub</sup> mice at 23 weeks. Blinded quantification of ten fields of view per biological sample.

Data information: In (B, D, G), each circle represents one individual mouse. Bars indicate mean. Mann–Whitney, *ns* not statistically different.

### **Appendix Figure S3.**

A - C. Western blots of LKB1 expression in MDCK cells expressing inducible shRNA against *Lkb1* (*Lkb1*-i1 and -i2) after tetracycline induction (Tet) (A, B). *Lkb1*-i1 rescue denotes *Lkb1*-i1 cells expressing degradation resistant full-length LKB1 (C).

D. Western blot of NPHP1 expression in *Nphp1*-i MDCK cells.

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H. qRT-PCR of *Strada* mRNA level in *Strada*-i MDCK cells (*n*=5).

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J. Western blot of KIF3a expression in *Kif3a*-i MDCK cells.

K. Western blot of IFT88 expression in *Ift88*-i MDCK cells.

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M. Western blot of IFT88 and LKB1 expression in *Ift88*-i *Lkb1*-i1 MDCK cells.

N. *Pkd1* gene structure with TALENs binding sites localisation and primer binding sites (P1-P3). The entire *Pkd1* gene was excised from MDCK cells in order to obtain PKD1 null cells.

O, P. Loss of *Pkd1* was validated by genomic PCR (O) and RT-PCR (P) in 2 different PKD1 null (-/-) MDCK cells in comparison to wild-type (+/+) or heterozygous (+/-) MDCK cells.

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R. Western blot of IFT88 expression in PKD1 null (KO1) MDCK cells expressing inducible shRNA against Ift88 (*Pkd1* KO1 Ift88-i) after tetracycline induction (+Tet).

S. Western blot of LKB1 expression in PKD1 null (KO1 and KO2) MDCK cells expressing inducible shRNA against Lkb1 (*Pkd1* KO1 or 2 Lkb1-i1) after tetracycline induction (Tet).

Data information: In (A - D, J - M, Q - S), representative western blot of at least 2 independent experiments. In (E - I), each circle represents one biological replicate. Bars indicate mean. Paired *t* test, \*\**P*<0.01, \*\*\**P*<0.001.

#### **Appendix Figure S4.**

A. Western blot of LKB1 expression in two different Anks3-i MDCK cell lines (Anks3-i1 and -i2) after tetracycline induction (+Tet).

B. Western blot of LKB1 expression in two different Nek7-i MDCK cell lines (Nek7-i1 and -i2).

C. Western blot of LKB1 expression in Nphp1-i MDCK cells.

D. Western blot of NPHP1, LKB1, NEK7 expression in two different PKD1 null MDCK cell lines (KO1 and 2).

E. qRT-PCR of *Lkb1* mRNA level in two different PKD1 null MDCK cell lines (KO1 and 2).

F. qRT-PCR of *Anks3* mRNA level in two different PKD1 null MDCK cell lines (KO1 and 2).

G. qRT-PCR of *Nek7* mRNA level in two different PKD1 null MDCK cell lines (KO1 and 2).

H. qRT-PCR of *Nphp1* mRNA level in two different PKD1 null MDCK cell lines (KO1 and 2).

I - L. Western blot of p-AMPK and total AMPK in two different *Anks3*-i MDCK cell lines (*Anks3*-i1 and -i2, I), two different *Nek7*-i MDCK cell lines (*Nek7*-i1 and -i2, J), *Nphp1*-i MDCK cells (K) and two different PKD1 null MDCK cell lines (KO1 and 2, L).

M. qRT-PCR of *Lkb1* mRNA level in kidneys from control and *iPkd1*<sup>ΔTub</sup> mice at 13.5 weeks.

N. qRT-PCR of *Anks3* mRNA level in kidneys from control and *iPkd1*<sup>ΔTub</sup> mice at 13.5 weeks.

O. qRT-PCR of *Nek7* mRNA level in kidneys from control and *iPkd1*<sup>ΔTub</sup> mice at 13.5 weeks.

P. qRT-PCR of *Nphp1* mRNA level in kidneys from control and *iPkd1*<sup>ΔTub</sup> mice at 13.5 weeks.

Data information: In (A - D, I - L), representative western blot of at least 2 independent experiments. In (E - H), each circle represents one biological replicate. Bars indicate mean. Paired *t* test, \**P*<0.05, \*\*\**P*<0.001, *ns* not statistically different. In (M - P), each circle represents one individual mouse. Bars indicate mean. Mann-Whitney, *ns* not statistically different.

## Appendix Figure S5.

A. Gating strategy to identify kidney myeloid cell subsets.

B, C. Representative flow cytometry analysis (B) and absolute numbers (C) of kidney leukocytes (CD45<sup>+</sup>DAPI<sup>-</sup>, black rectangles) from control and *Lkb1*<sup>ΔTub</sup> mice at 10 weeks.

D, E. Representative flow cytometry analysis (D) and absolute numbers (E) of kidney T cells (CD45<sup>+</sup>DAPI<sup>-</sup>CD3<sup>+</sup>, gated region) from control and *Lkb1*<sup>ΔTub</sup> mice at 10 weeks.

F, G. Representative flow cytometry analysis (F) and absolute numbers (G) of kidney B cells (CD45<sup>+</sup>DAPI<sup>-</sup>B220<sup>+</sup>, black rectangles) from control and *Lkb1*<sup>ΔTub</sup> mice at 10 weeks.

H, I. Representative flow cytometry analysis (H) and absolute numbers (I) of kidney neutrophils (CD45<sup>+</sup>DAPI<sup>-</sup>Lin<sup>-</sup>Ly6G<sup>+</sup>CD11b<sup>hi</sup>, black rectangles) from control and *Lkb1*<sup>ΔTub</sup> mice at 10 weeks.

J, K. Representative flow cytometry analysis (J) and absolute numbers (K) of kidney macrophages (F4/80<sup>hi</sup>CD11b<sup>+</sup>, red rectangles) from control and *Lkb1*<sup>ΔTub</sup> mice at 10 weeks.

L. Percentage of CCR2<sup>+</sup> macrophages in F4/80<sup>hi</sup>CD11b<sup>+</sup> population from control and *Lkb1*<sup>ΔTub</sup> mice at 10 weeks.

Data information: In (B, D, F, H, J), numbers represent percentage of cells per gated region. In (C, E, G, I, K, L), each circle represents one individual mouse. Bars indicate mean. Mann-Whitney, \**P*<0.05, \*\**P*<0.01, *ns* not statistically different.

## Appendix Figure S6.

A, B. Representative flow cytometry analysis (A) and absolute numbers (B) of kidney leukocytes (CD45<sup>+</sup>DAPI<sup>-</sup>, black rectangles) in control, *iPkd1*<sup>ΔTub</sup> and *iPkd1*<sup>ΔTub</sup>; *iKif3a*<sup>ΔTub</sup> mice at 12 weeks.

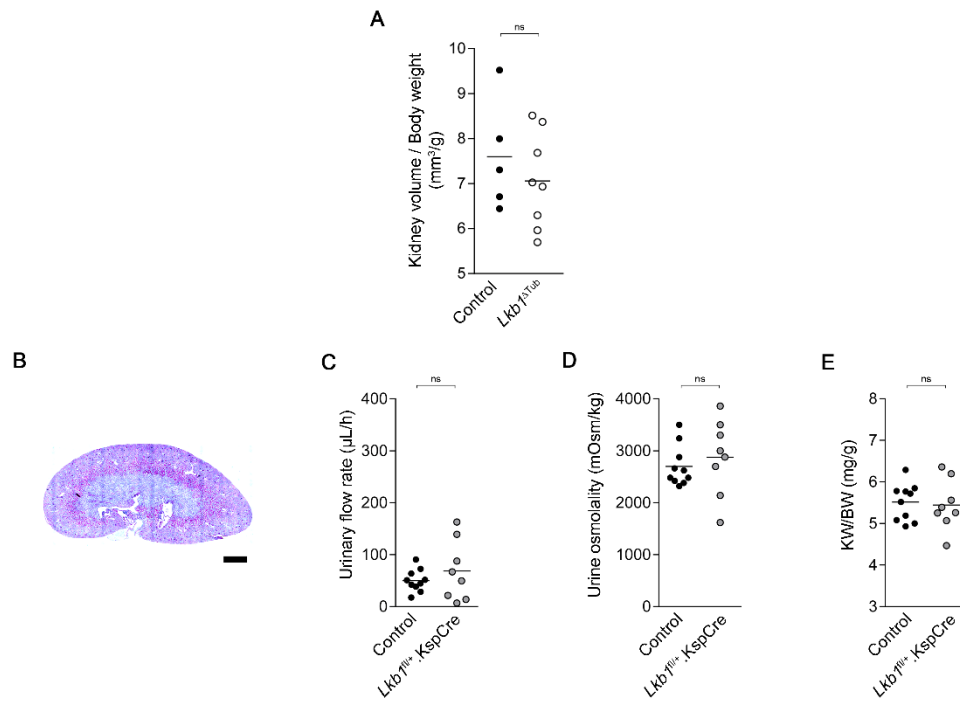
C, D. Representative flow cytometry analysis (C) and absolute numbers (D) of kidney T cells (CD45<sup>+</sup>DAPI<sup>-</sup>CD3<sup>+</sup>, gated region) in control, *iPkd1*<sup>ΔTub</sup> and *iPkd1*<sup>ΔTub</sup>; *iKif3a*<sup>ΔTub</sup> mice at 12 weeks.

E, F. Representative flow cytometry analysis (E) and absolute numbers (F) of kidney B cells (CD45<sup>+</sup>DAPI<sup>-</sup>B220<sup>+</sup>, black rectangles) in control, *iPkd1*<sup>ΔTub</sup> and *iPkd1*<sup>ΔTub</sup>; *iKif3a*<sup>ΔTub</sup> mice at 12 weeks.

G, H. Representative flow cytometry analysis (G) and absolute numbers (H) of kidney neutrophils (CD45<sup>+</sup>DAPI<sup>-</sup>Lin<sup>-</sup>Ly6G<sup>+</sup>CD11b<sup>hi</sup>, black rectangles) in control, *iPkd1*<sup>ΔTub</sup> and *iPkd1*<sup>ΔTub</sup>; *iKif3a*<sup>ΔTub</sup> mice at 12 weeks.

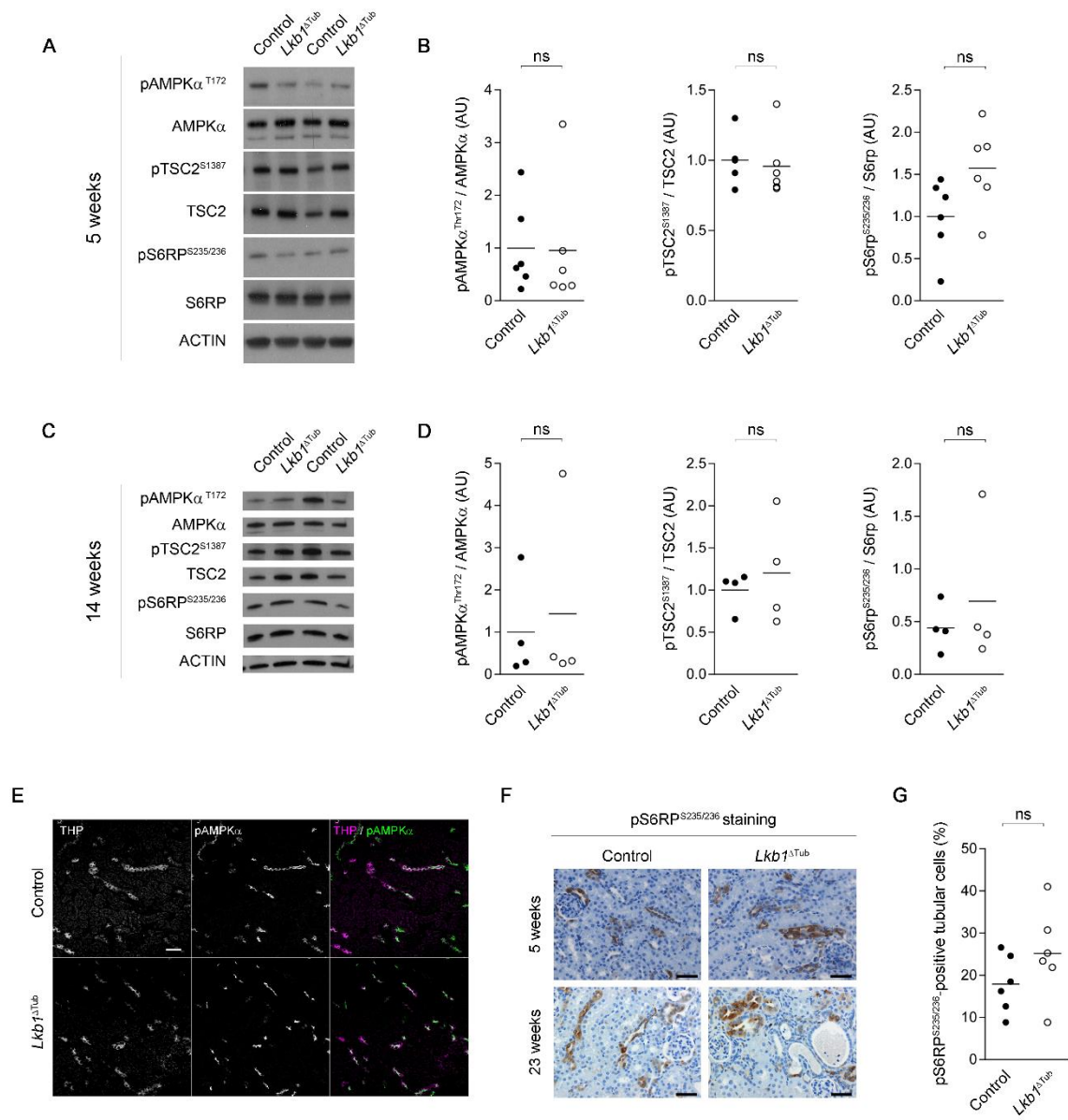
Data information: In (A, C, E, G), numbers represent percentage of cells per gated region. In (B, D, F, H), each dot represents one individual mouse. Bars indicate mean. ANOVA followed by Tukey-Kramer test, \*\**P*<0.01, *ns* not statistically different.

Appendix Figure S1

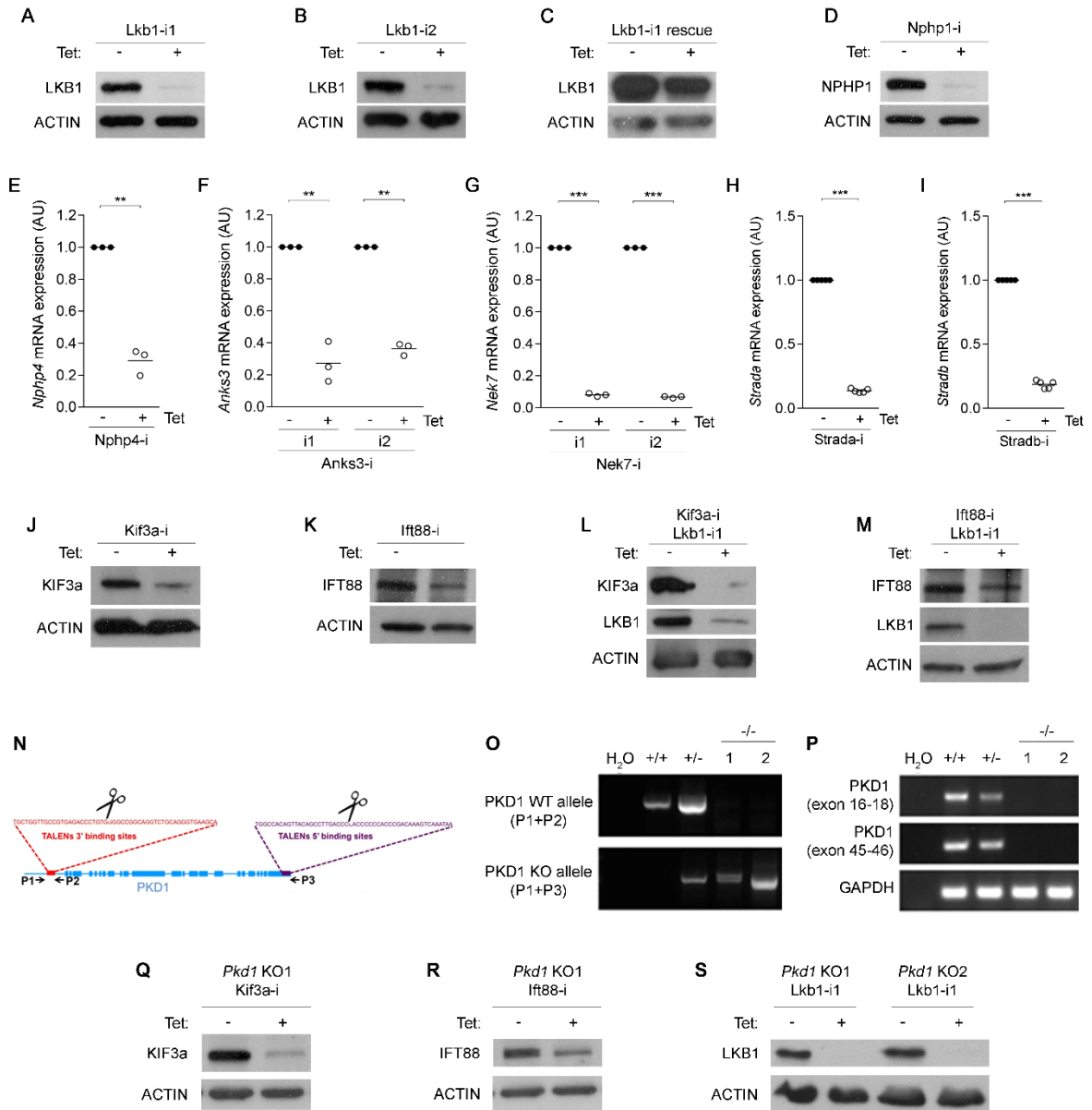




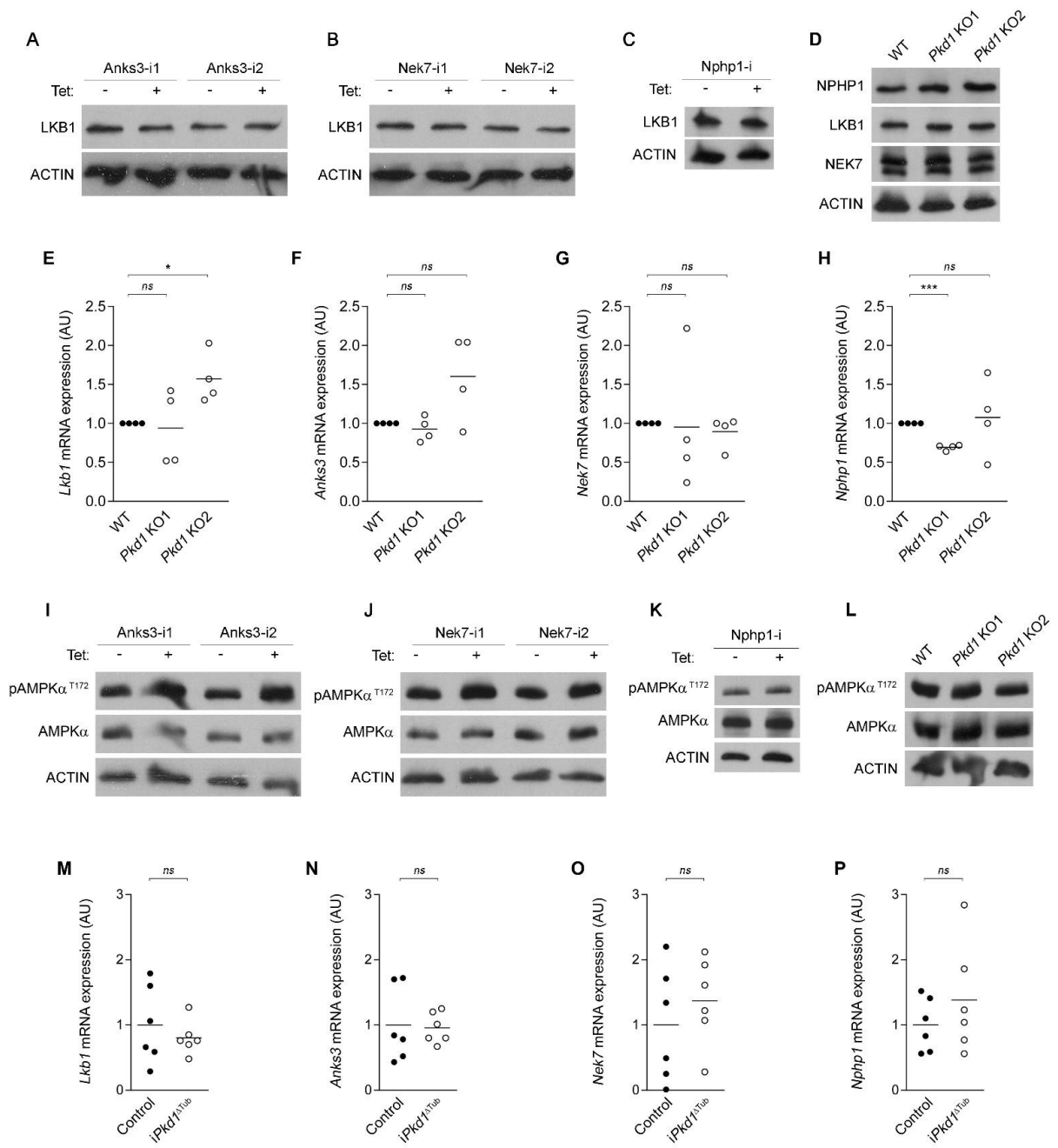
Appendix Figure S2



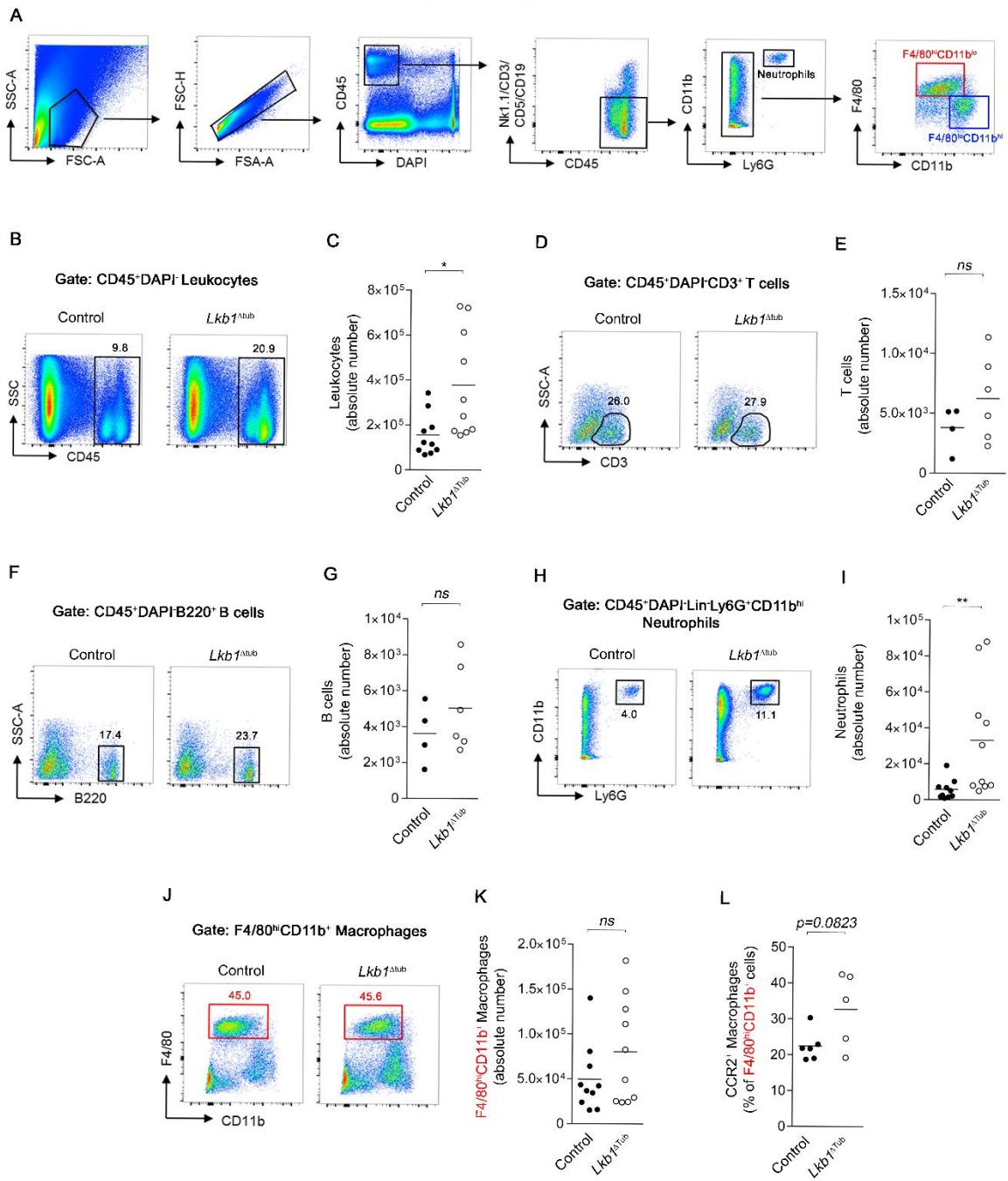
Appendix Figure S3



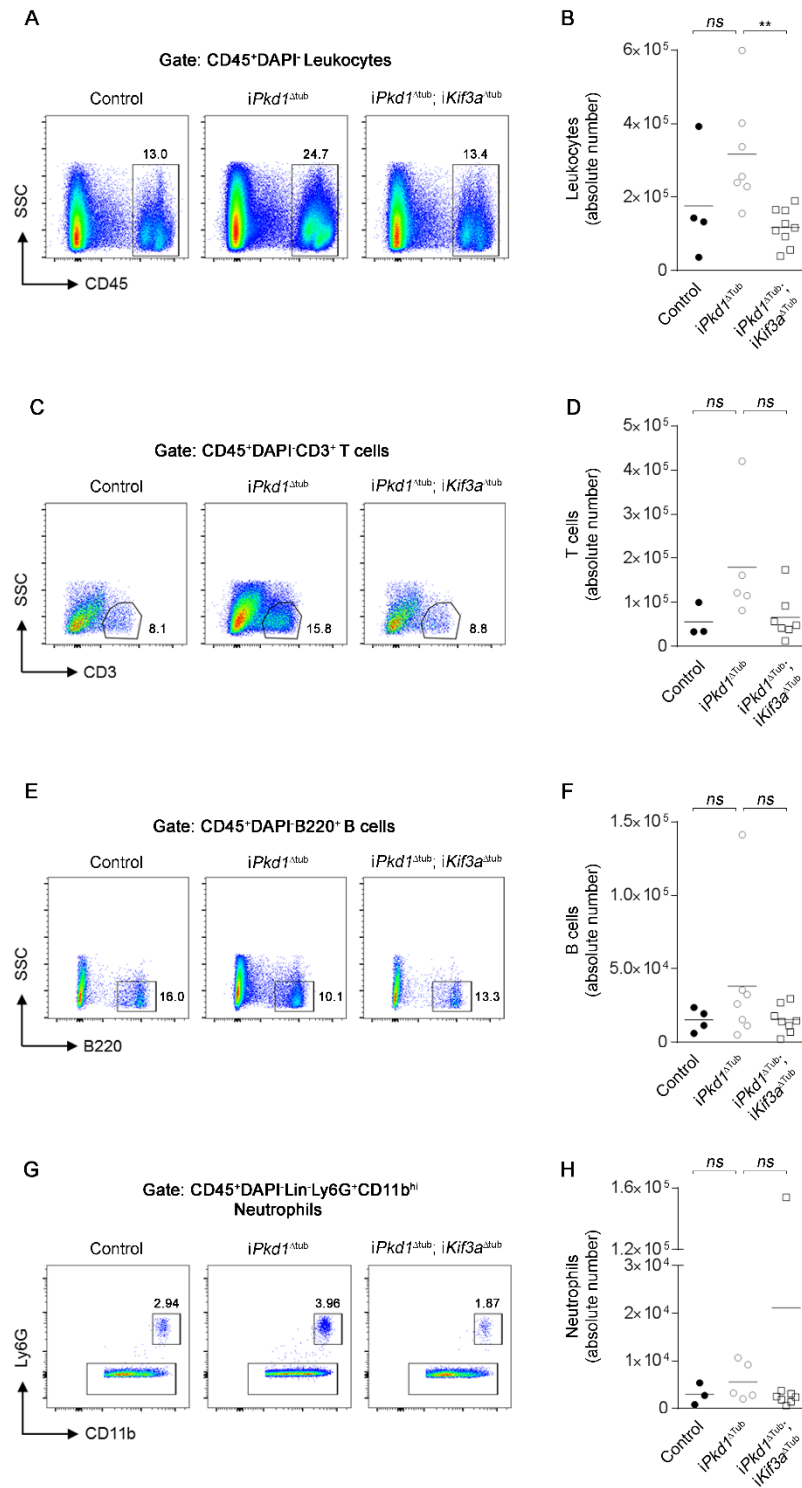
Appendix Figure S4



Appendix Figure S5



Appendix Figure S6



Appendix Table S1: Primer list					
a: primer used for cloning					
Construct	PMID	Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	
FLAG.MO25a pcDNA6		Dog	CGCGGGACGCGTATGCCGTTCCCATTTGGGAAGTC	CGCGGGGCGGCCGCGCTTAGGCTTCTTGCTGAGC	
FLAG.MO25b pcDNA6		Dog	CGCGGGACGCGTATGAAAAAATGCCTTTGTTTAGTAAATC	CGCGGGGCGGCCGCGCTCAAGGGCTGCTCCTC	
Venus.LKB1 or Flag.LKB1 pLXSN		Human	CGCGGGACGCGTATGGAGGTGGTGGACCCGCAGCAGCTGGGC	ATAGTTTAGCGGCCGCGCTCACTGCTGCTTGCAGGCCGACAGCCGGCG	
STRADa.Venus pLXSN		Human	CGCGGGACGCGTGCCACCATGTCTATTCTTGTAAAGTAAACCAGAG	CGCGGGGCGGCCGCGCGAACTCCCAATCGTCCACCTCCAGCTCTTC	
STRADb.Venus pLXSN		Human	CGCGGGACGCGTGCCACCATGTCTCTTTTGATTGCTTCTGC	CGCGGGGCGGCCGCGCGAATTCACAGTATGAGTCTTTTTC	
b: primer used for qRT-PCR					
Gene name	PMID	Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	
Anks3		Dog	GACAGAGAGGTCTCCCTATTCA	CAGGTACTTCAGACACCCAATC	
Ccl2		Dog	CTGCTGCTATACACTACCAATA	TCAGCACAGATCTCCTTGTTTAG	
Gapdh		Dog	CATGTTTGTGATGGGCGTGAACCA	TTTGGCTAGAGGAGCCAAGCAGTT	
Hprt		Dog	AATGTCCTTGATTGTTGAGGATAT	ACAAAGTCAGGTTTATAGCC	
Nek7		Dog	TCTGGTCTCTTGGCTGTCTA	GGGTAGTCACACTGTTCTATCTTC	
Nphp1	19755384	Dog	AGCAGGAGGGGAAGAAGT	TGGAAGAGTGTGGAAGGC	
Nphp4	19755384	Dog	ATAGTTCGCTGGGCTGTT	CTGGCAGGGGTTACTTTA	
Sdha		Dog	TGGGTCCATCCATCGCATA	GAAGTAGGTGCGCCATAACC	
Strada		Dog	GCCATATCGAGCCACCTTTAT	GTAAGCAATCGCCAGTTCATTC	
Stradb		Dog	CTAGAGCCAAGGATGTGATCTG	CAGTGTTCCTGTGGGAGTATG	
Anks3		Mouse	GCAGTGAAGCCTCCATAGAAA	TTGCCGTGACGACTCTTATTC	
Ccl2		Mouse	AGTAGGCTGGAGAGCTACAA	GTATGTCGGACCCATTCTTC	
Col1a1		Mouse	GCCGCAAAGAGTCTACATGTCTAG	TGGCAGATACAGATCAAGCATACC	
Col3a1		Mouse	GGACCAGCAGGAACATAATGGTAT	GTTCTCCAGGTGATCCATCTTT	
Col4a1		Mouse	GTCTGGCTTCTGCTGCTCTTC	CCTTCACGCCATGACAGTCA	
Gapdh		Mouse	TGCACCACCAACTGCTTAG	TGGATGCAGGGATGATGTT	
Ilgam		Mouse	CTTCCAGGGCAGGAGTCGTAT	CAATGGAGCTGCCCACAAT	
Lkb1	22145035	Mouse	CCTGCAAGCAGCAGTGAC	CCAACGTCCGAAGTGAG	
Nek7		Mouse	GTCTCTTGGCTGTCTGCTATATG	GGGTAGTCACACTGCTCTATCT	
Nphp1		Mouse	GTGAGCATCAGCAGGAAAGA	CTTCTTCCTCTCCACCAGTTTC	
Rpl13		Mouse	CTCATCTGTTCCCCAGGAA	GGGTGGCCAGCTTAAGTTCTT	
Sdha		Mouse	AGAACATCAGAACTACGCCTAAACATG	CCATTCCCCTGTGCAATGTCT	
c: primer used for genomic PCR					
Gene name		Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	
P1		Dog	TGGAGCTGTTAGGTGTCC		
P2		Dog		CCACTGCCTGCCCTTCGA	
P3		Dog		CCCTGGAGGGCTCTCTGC	
Ccl2		Mouse	AACCACCTCAAGCACTTCTG	GCTTTGCAGTTTCCCTCAAG	
Cre		Mouse	GCATAACCAGTGAAACAGCATTGCTG	GGACATGTTCAAGGATCGCCAGGCG	
Kif3a	10220415	Mouse	TCTGTGAGTTTGTGACCAGCC	AGGGCAGACGGAAGGGTGG	
Lkb1		Mouse	ATCGGAATGTGATCCAGCTT	ACGTAGGCTGTGCAACCTCT	
rtTA	18724376	Mouse	CCATGTCTAGACTGGACAAGA	CTCCAGGCCACATATGATTAG	
Pkd1		Mouse	CCTGCCTTGCTCTACTTTCC	AGGGCTTTTCTGCTGGTCT	
d: primer used for RT-PCR					
Gene name		Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	
Gapdh		Dog	CATGTTTGTGATGGGCGTGAACCA	TTTGGCTAGAGGAGCCAAGCAGTT	
Pkd1 (exon 16-18)		Dog	ACACCTTCAACCTGACTGTGTGGA	TTGGAACCTCGCTGCCAGTTATCA	
Pkd1 (exon 45-46)		Dog	TTCTGCTGGTCTCCTCCTGT	GCAGAAAGAGCTCCACCATC	