

## **APPENDIX**

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## Appendix Figure legends

### Appendix Figure S1.

A. Magnetic resonance renal volume to body weight ratio from control and *Lkb1 $\Delta$ Tub* 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 $\Delta$ Tub* 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 $\Delta$ Tub* 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 $\Delta$ Tub* animals. Representative images of 7 mice/group. pAMPK $\alpha$  is present at the apical surface of THP positive tubules in both control and *Lkb1 $\Delta$ Tub* 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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M. Western blot of IFT88 and LKB1 expression in *Ift88*-i *Lkb1*-i1 MDCK cells.

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Q. Western blot of KIF3a expression in PKD1 null (KO1) MDCK cells expressing inducible shRNA against Kif3a (*Pkd1* KO1 Kif3a-i) after tetracycline induction (+Tet).

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.

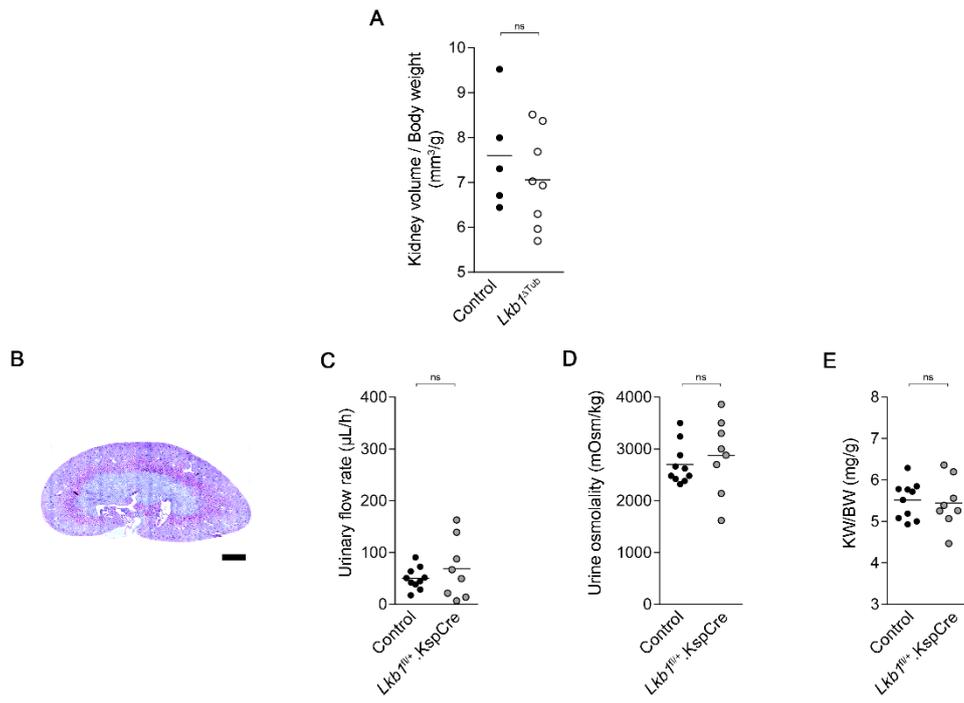
C, D. Representative flow cytometry analysis (C) and absolute numbers (D) of kidney T cells (CD45<sup>+</sup>DAPI<sup>-</sup>CD3<sup>+</sup>, gated region) in control, *iPkd1*<sup>ΔTub</sup> and *iPkd1*<sup>ΔTub</sup>; *iKif3a*<sup>ΔTub</sup> mice at 12 weeks.

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.

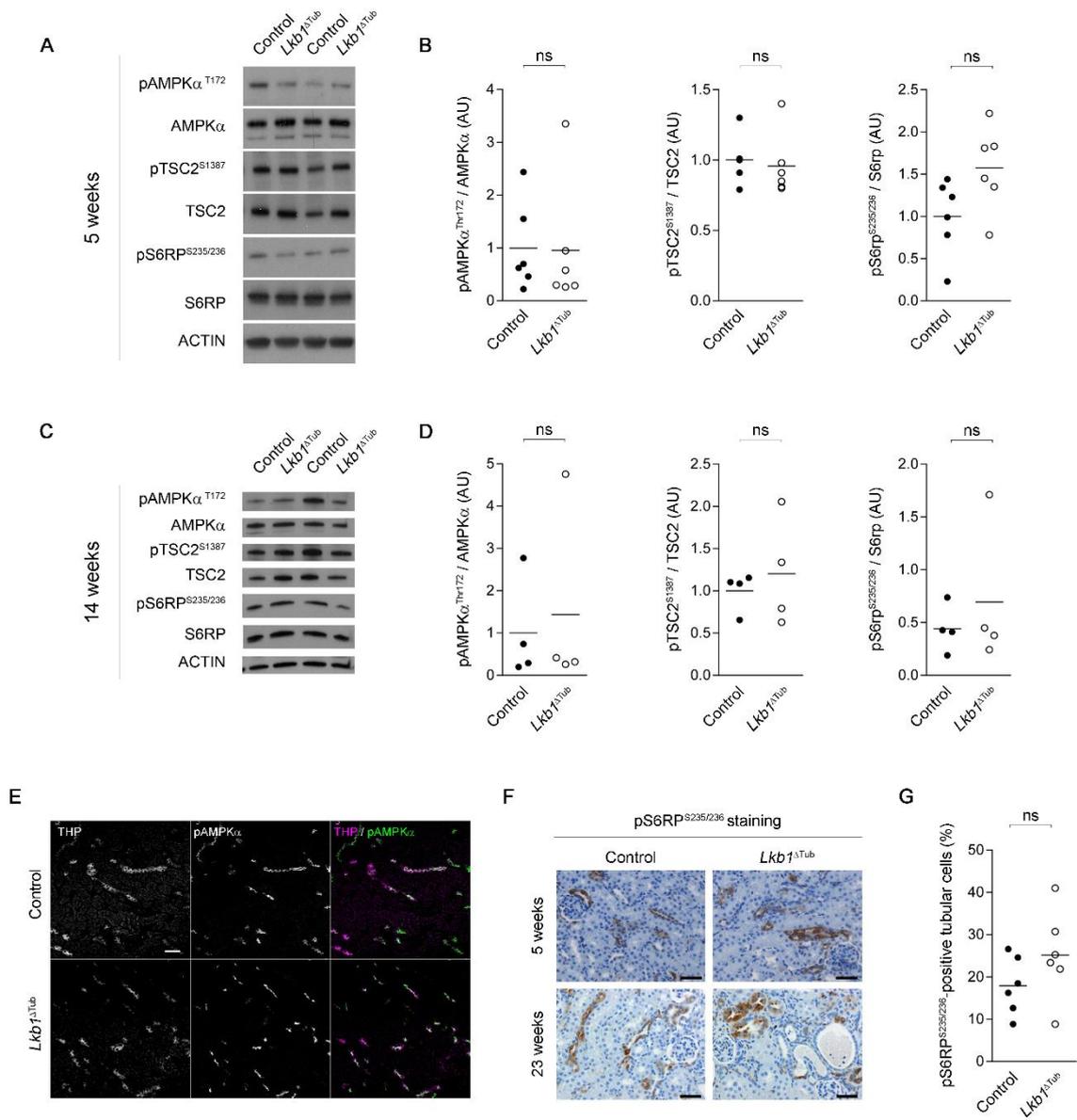
G, H. Representative flow cytometry analysis (G) and absolute numbers (H) of kidney neutrophils (CD45<sup>+</sup>DAPI<sup>-</sup>Lin<sup>-</sup>Ly6G<sup>+</sup>CD11b<sup>hi</sup>, black rectangles) in control, *iPkd1*<sup>ΔTub</sup> and *iPkd1*<sup>ΔTub</sup>; *iKif3a*<sup>ΔTub</sup> mice at 12 weeks.

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.

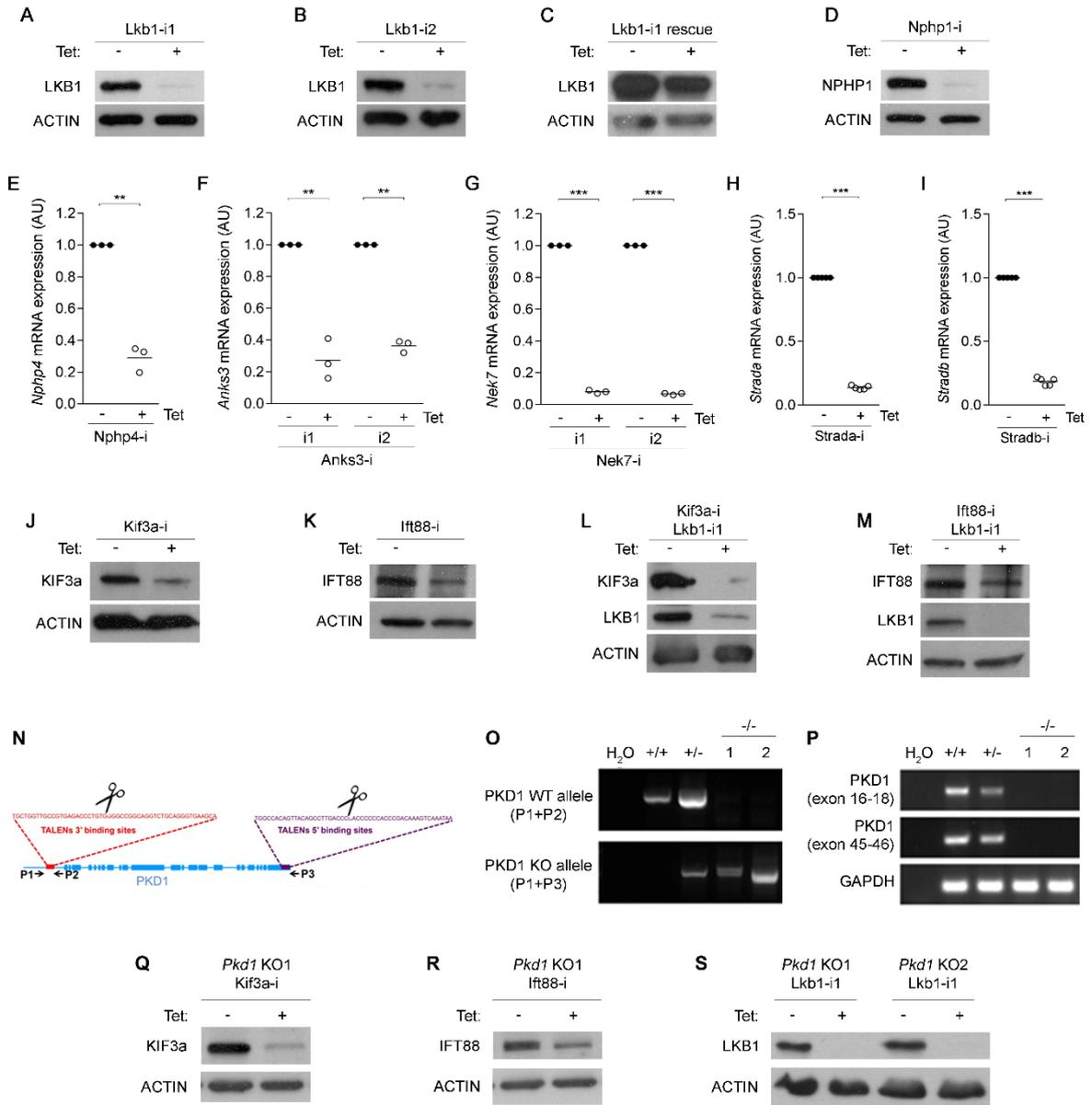
# Appendix Figure S1



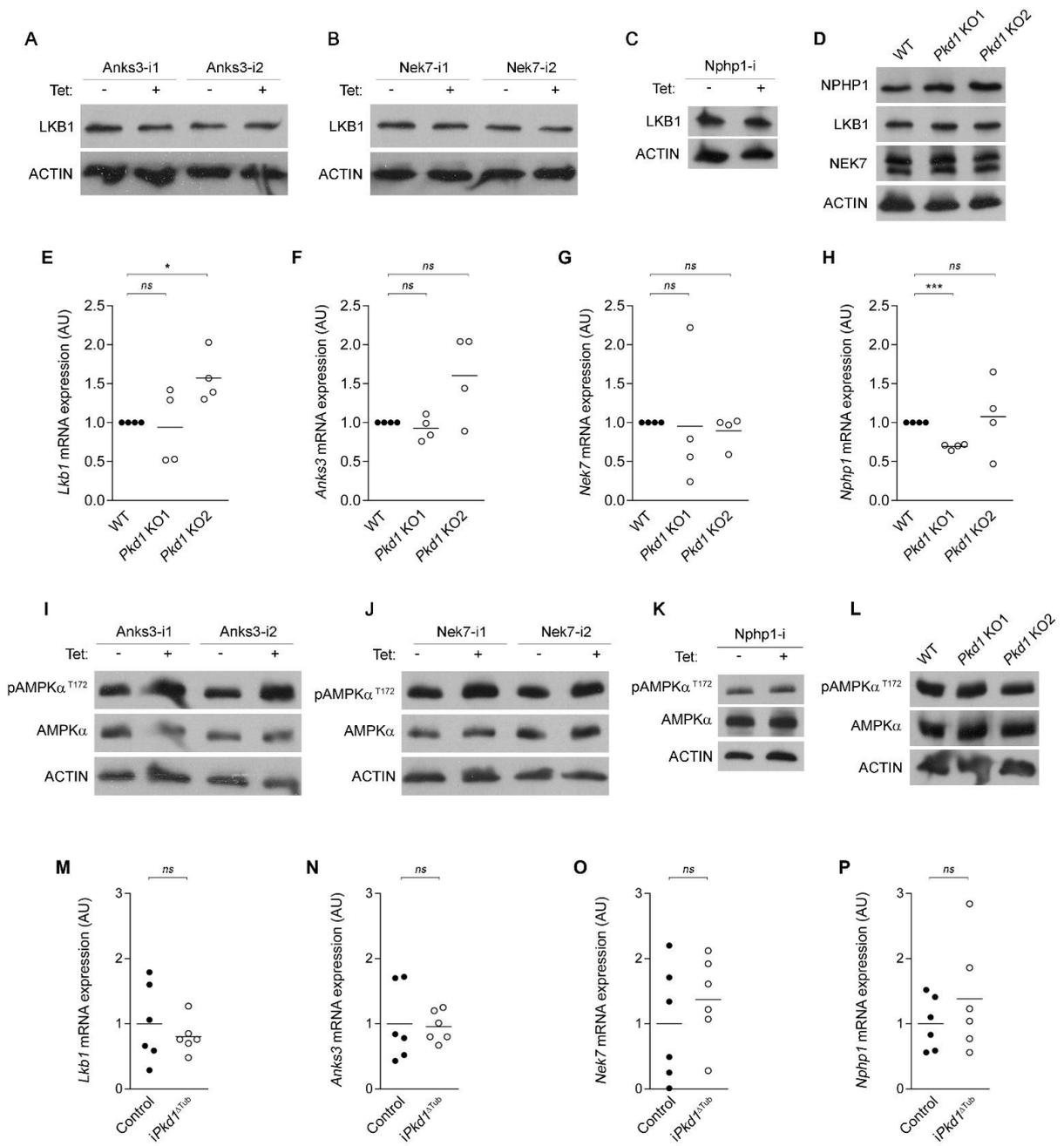
## Appendix Figure S2



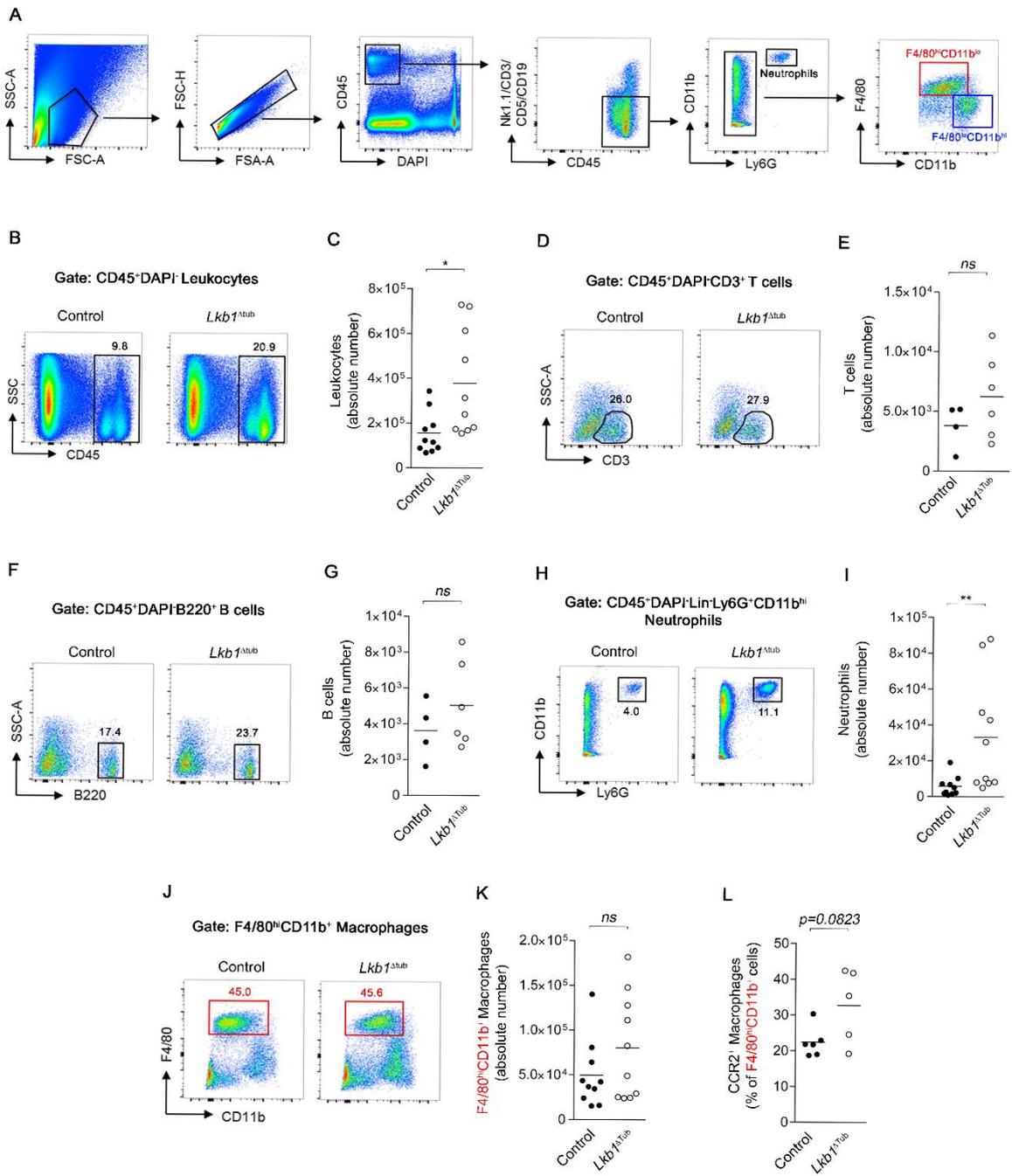
### Appendix Figure S3



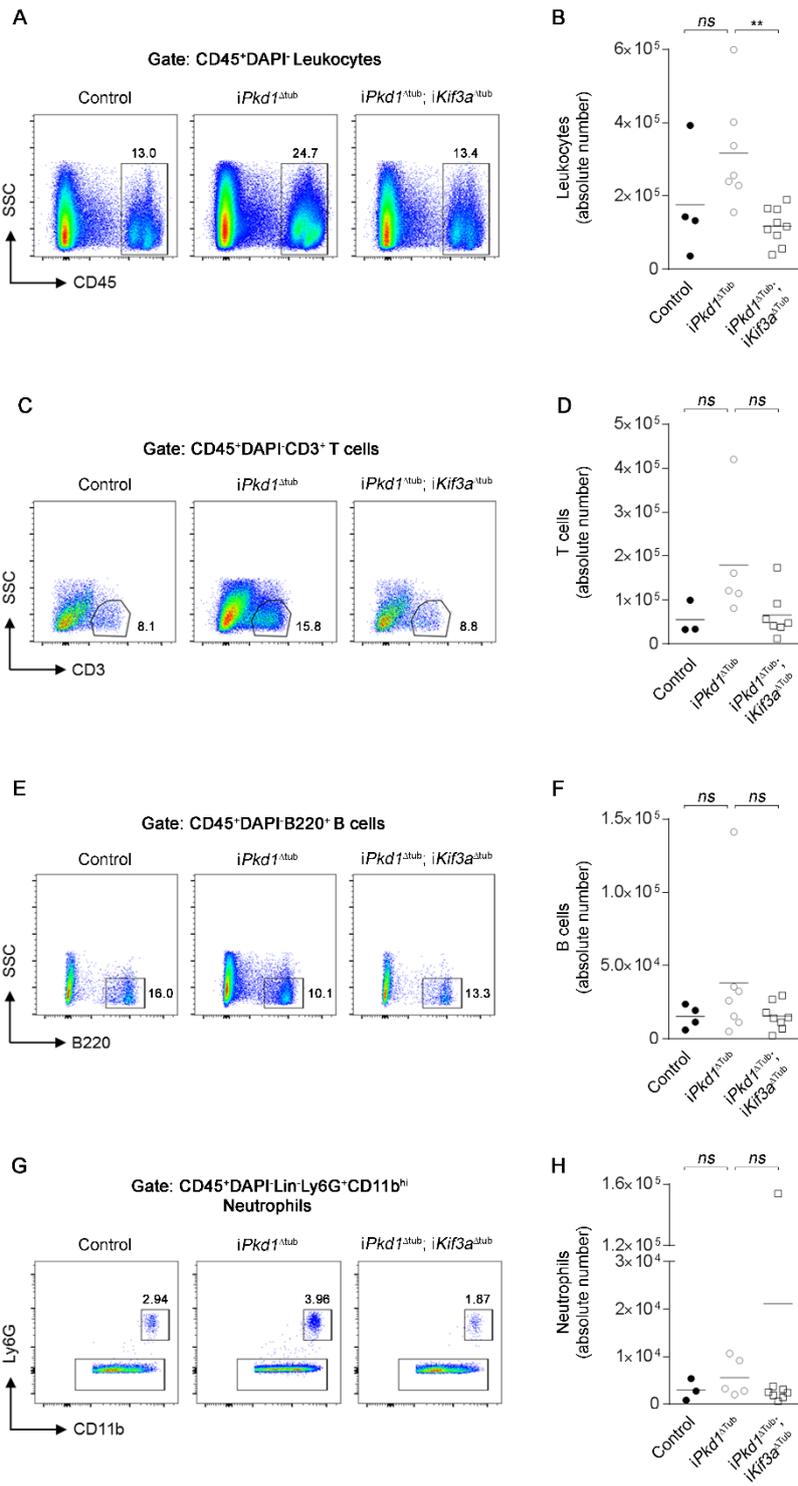
### Appendix Figure S4



### Appendix Figure S5



## Appendix Figure S6



Appendix Table S1: Primer list				
<b>a: primer used for cloning</b>				
Construct	PMID	Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
FLAG.MO25a pcDNA6		Dog	CGCGGGACGCGTATGCCGTTCCCATTTGGGAAGTC	CGCGGGGCGCCGCCCTTAGGCTTCTTGCTGAGC
FLAG.MO25b pcDNA6		Dog	CGCGGGACGCGTATGAAAAAATGCCTTTGTITAGTAAATC	CGCGGGGCGCCGCCCTAAGGGCTGCTCCTC
Venus.LKB1 or Flag.LKB1 pLXSN		Human	CGCGGGACGCGTATGGAGGTGGTGGACCCGCAGCAGCTGGGC	ATAGTTTAGCGGGCCCTCACTGTCTCTTGACAGGCCAGAGCCGGCG
STRADa.Venus pLXSN		Human	CGCGGGACGCGTGCCACCATGTCAATTTCTTGAAGTAAACCAGAG	CGCGGGGCGCCGCCGAACTCCCAATCGTCCACCTCCAGCTCTTC
STRADb.Venus pLXSN		Human	CGCGGGACGCGTGCCACCATGTCTCTTTGGATTGCTTCTGC	CGCGGGGCGCCGCCGAATTCCCAAGTATGAGCTTTTTTC
<b>b: primer used for qRT-PCR</b>				
Gene name	PMID	Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>Anks3</i>		Dog	GACAGAGAGGTCTCCCTATTCA	CAGGTACTTCAGACACCCCAATC
<i>Ccl2</i>		Dog	CTGCTGTATACACTCACCAATA	TCAGCACAGATCTCCTTGTTTAG
<i>Gapdh</i>		Dog	CATGTTTGTGATGGGCGTGAACCA	TTTGGCTAGAGGAGCCAAGCAGTT
<i>Hprt</i>		Dog	AATGTCITGATTTGTTGAGGATAT	ACAAAGTCAGGTTTATAGCC
<i>Nek7</i>		Dog	TCTGGTCTCTGGCTGTCTA	GGGTAGTCACACTGTTCTATCTTC
<i>Nphp1</i>	19755384	Dog	AGCAGGAGGGGAAGAAGT	TGGAAGAGTGTGGAAGGC
<i>Nphp4</i>	19755384	Dog	ATAGTTCGCTGGGCTGTT	CTGGCAGGGGTTACTTTA
<i>Sdha</i>		Dog	TGGTCCATCCATCGCATA	GAAGTAGGTGCGCCCATAAACC
<i>Strada</i>		Dog	GCCATATCGAGCCACCTTTAT	GTAAGCAATCGCCAGTTCATTC
<i>Stradb</i>		Dog	CTAGAGCCAAGGATGTGATCTG	CAGTGTTCCTGTGGGAGTATG
<i>Anks3</i>		Mouse	GCAGTGAAGCCTCCATAGAAA	TTGCCGTGACGACTCTTATTC
<i>Ccl2</i>		Mouse	AGTAGGCTGGAGAGCTACAA	GTATGTCGGACCCATCCCTTC
<i>Col1a1</i>		Mouse	GCCGCAAAGAGTCTACATGTCTAG	TGGCAGATACAGATCAAGCATACC
<i>Col3a1</i>		Mouse	GGACCAGCAGGAACCTAATGGTAT	GTTCTCCAGGTGATCCATCTTT
<i>Col4a1</i>		Mouse	GTCTGGCTTCTGCTGCTCTTC	CCTTCACGCCATGACAGTCA
<i>Gapdh</i>		Mouse	TGCACCACCAACTGCTTAG	TGGATGCAGGGATGATGTT
<i>Ilgam</i>		Mouse	CTTCCAGGGCAGGAGTCTGAT	CAATGGAGCTGCCACAAT
<i>Lkb1</i>	22145035	Mouse	CCTGCAAGCAGCAGTGAC	CCAACGTCCCCGAAGTGA
<i>Nek7</i>		Mouse	GTCTCTGGCTGTCTGTATATG	GGGTAGTCACACTGCTCTATCT
<i>Nphp1</i>		Mouse	GTGACATCAGCAGGAAAGA	CTTCTTCCCTCCCAACCTTC
<i>Rpl13</i>		Mouse	CTCATCTGTTCCCAAGGAA	GGGTGGCCAGCTTAAGTCTT
<i>Sdha</i>		Mouse	AGAACATCAGAACTACGCCTAAACATG	CCATTCCCTGTGCAATGTCT
<b>c: primer used for genomic PCR</b>				
Gene name		Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>P1</i>		Dog	TGGAGCTGTTAGGTGTCC	
<i>P2</i>		Dog		CCACTGCCTGCCCTTCGA
<i>P3</i>		Dog		CCCTGGAGGGCTCTCTGC
<i>Ccl2</i>		Mouse	AACCACCTCAAGCACTTCTG	GCTTTCAGTTTCCCTCAAG
<i>Cre</i>		Mouse	GCATAACCAGTGAACAGCATTGCTG	GGACATGTTCAAGGATCGCCAGGCG
<i>Kif3a</i>	10220415	Mouse	TCTGTGAGTTTGTGACCAGCC	AGGGCAGACGGAAGGGTGG
<i>Lkb1</i>		Mouse	ATCGGAATGTGATCCAGCTT	ACGTAGGCTGTGCAACCTTC
<i>rtTA</i>	18724376	Mouse	CCATGTCTAGACTGGACAAGA	CTCCAGGCCACATATGATTAG
<i>Pkd1</i>		Mouse	CCTGCCTTGCTCTACTTTCC	AGGGCTTTTTCTTGCTGGTCT
<b>d: primer used for RT-PCR</b>				
Gene name		Specie	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>Gapdh</i>		Dog	CATGTTTGTGATGGGCGTGAACCA	TTTGGCTAGAGGAGCCAAGCAGTT
<i>Pkd1</i> (exon 16-18)		Dog	ACACCTTCAACCTGACTGTGTGGA	TTGGAACCTCGCTGCAGTTATCA
<i>Pkd1</i> (exon 45-46)		Dog	TTCTGCTGGTCTCCCTCTGT	GCAGAAAAGAGCTCCACCATC