Supplementary Material

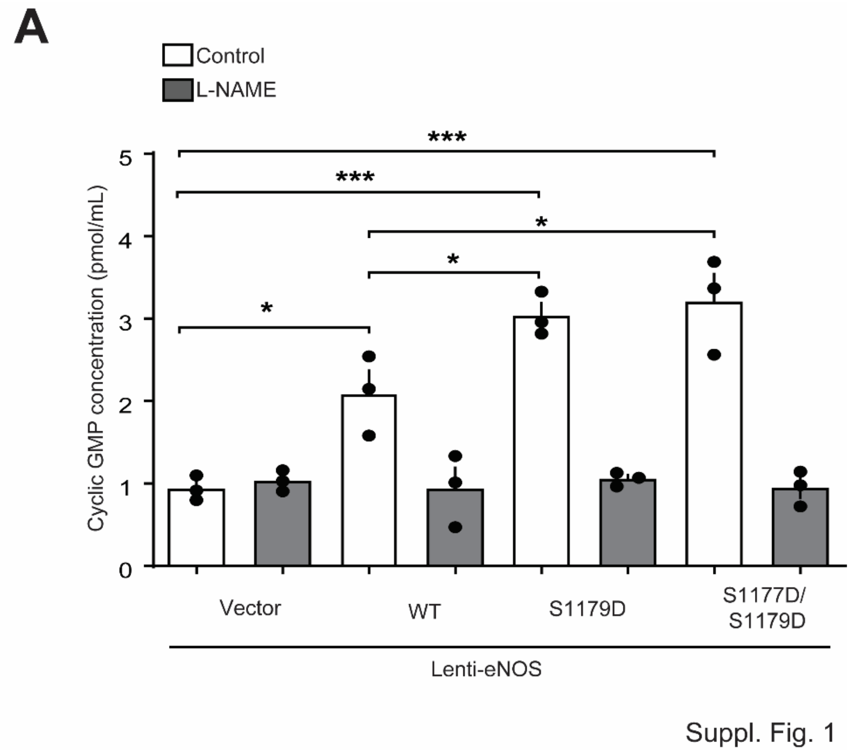
Manuscript

Protein kinase N2 mediates flow-induced eNOS activation and vascular tone regulation

(Jin et al.)

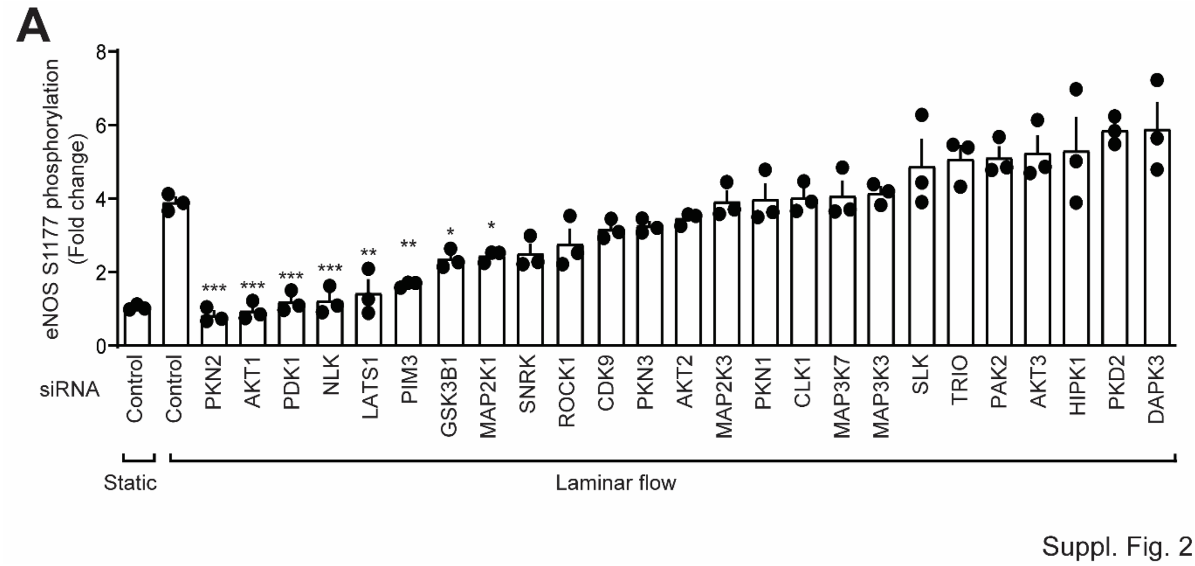
Supplementary Figures

Suppl. Fig. 1



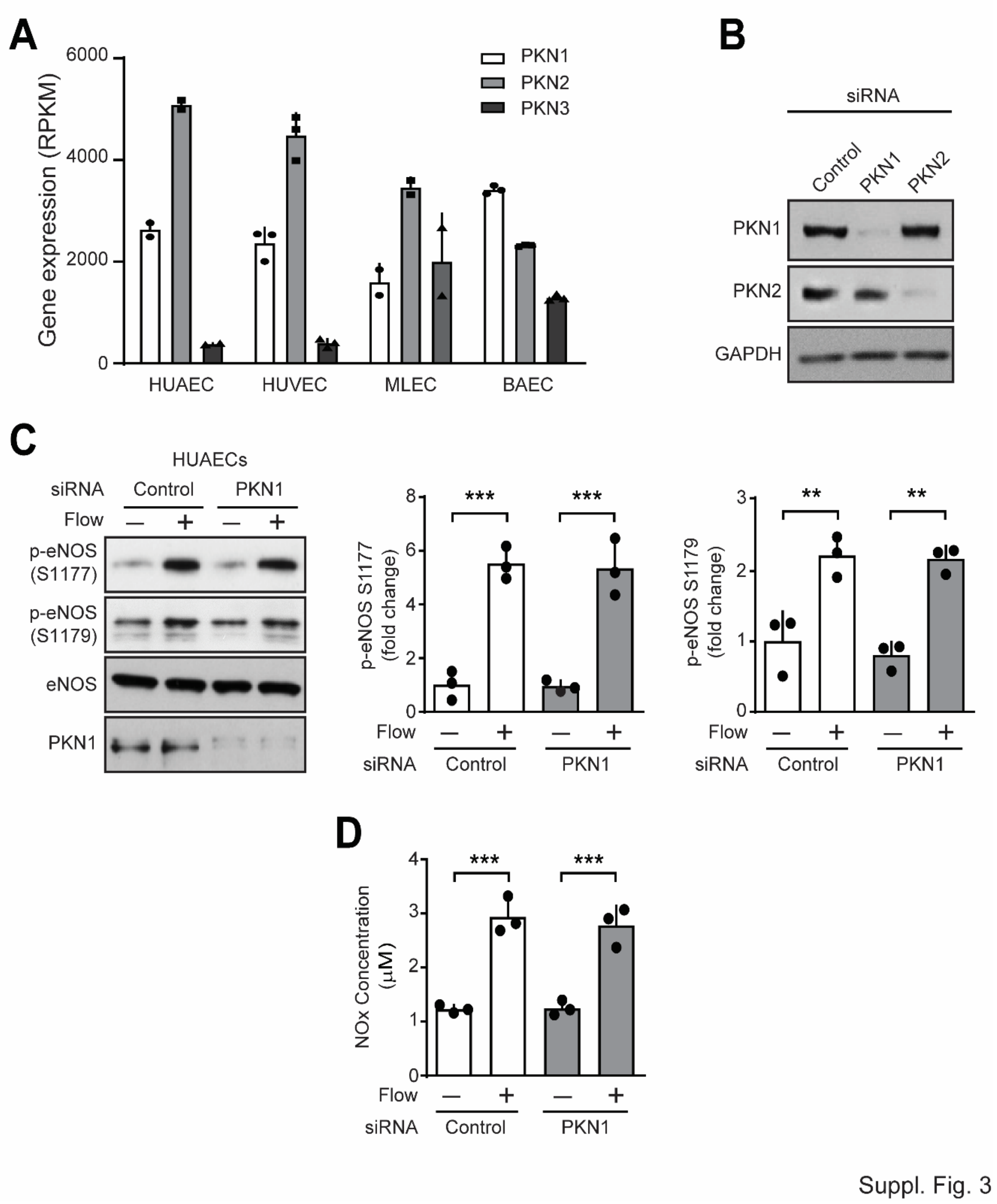
Supplementary Figure 1. Effect of phosphomimetic mutants of eNOS on NO formation determined by analyzing cGMP levels. Wild-type (WT) human eNOS or the indicated eNOS mutants were expressed by lentiviral transduction after siRNA-mediated eNOS knock-down in HUAECs. Thereafter cells were kept in the absence or presence of 1 µM L-NAME, and NO formation was indirectly determined after exposure of RFL-6 cells to supernatants of HUAECs and determination of cGMP levels in RFL-6 cells using an ELISA assay. Data represent mean ± SEM; *, $P \le 0.05$; ***, $P \le 0.001$ (two-way ANOVA with Bonferroni's post-hoc test).

Suppl. Fig. 2



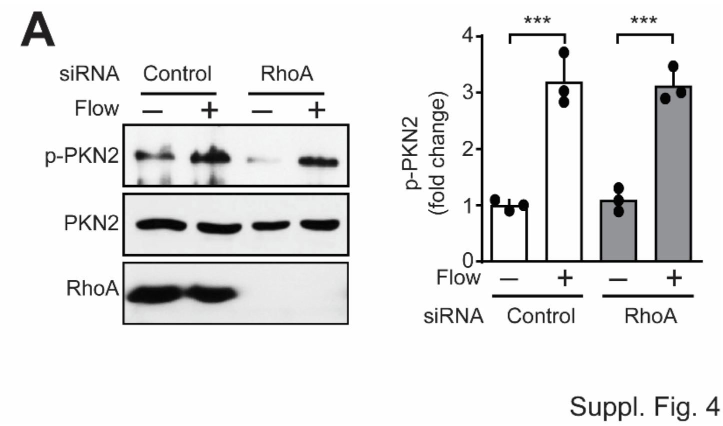
Supplementary Figure 2. Effect of knock-down of different protein kinases on flow-induced phosphorylation of human eNOS at serine 1177. (**A**) HUAECs were transfected with control siRNA or siRNAs directed against the indicated protein kinases and were exposed to laminar flow (15 dynes/cm^2) for 15 minutes. Shown is the ratio of flow-induced phosphorylation of eNOS at serine 1177 in cells transfected with control siRNA and siRNA against a particular protein kinase. The plot shows the ranked ratio of 3 independent experiments. Data represent the mean ± SEM; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, (two-way ANOVA with Bonferroni's post hoc test).

Suppl. Fig. 3



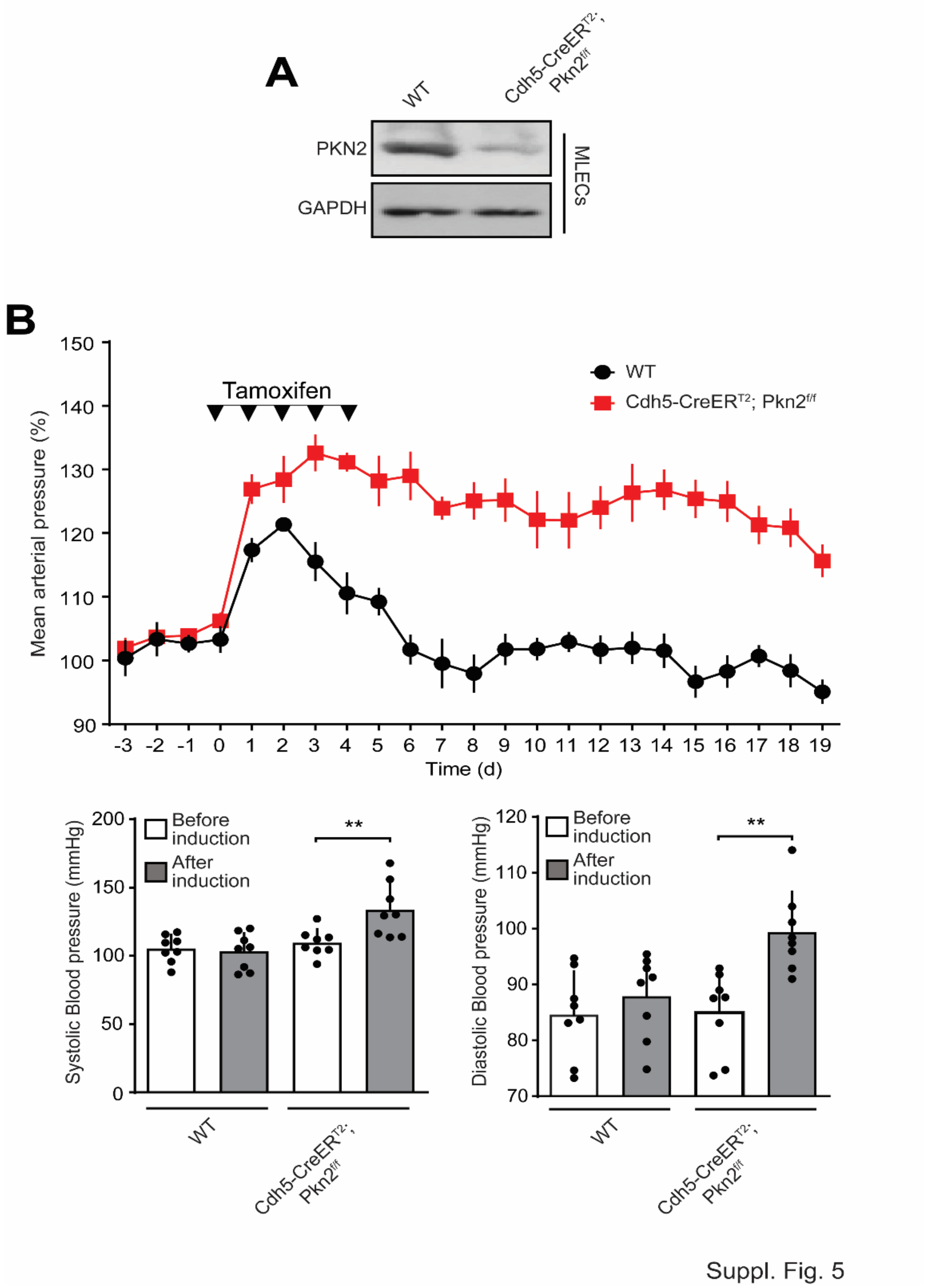
Supplementary Figure 3. Comparison of PKN isoform expression in endothelial cells, validation of PKN1 and PKN2 knockdown efficiency and role of PKN1 in flow induced effects. (**A**) PKN isoform expression was assessed by RNA sequencing in various endothelial cells. HUAEC, human umbilical artery endothelial cell; HUVEC, human umbilical vein endothelial cell; MLEC, mouse lung endothelial cell; BAEC, bovine aortic endothelial cells. RPKM, reads per kilo base per million mapped reads. (**B**) Validation of efficiency of knockdown of PKN1 and PKN2 using specific siRNAs by immunoblotting. (**C**,**D**) HUAECs were transfected with control siRNA or siRNA against PKN1 and were exposed to laminar flow (15 dynes/cm^2). Total and phosphorylated eNOS as well as PKN1 were determined by immunoblotting (C), or nitrate/nitrite levels were determined in the supernatants (D). Bar diagrams show the densitometric evaluation of blots (n=3 independent experiments). Data represent the mean ± SEM; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, (one-way ANOVA, with Tukey's post-hoc test (C,D)).

Suppl. Fig. 4



Supplementary Figure 4. RhoA is not involved in flow-induced PKN2 activation. (**a**) HUAECs were transfected with control siRNA or siRNAs directed against RhoA and were then exposed to laminar flow (15 dynes/cm^2). Total and phosphorylated PKN2 as well as RhoA protein levels were determined by immunoblotting. The bar diagram shows the densitometric evaluation of blots (n=3 independent experiments). Data represent mean ± SEM; ***, P ≤ 0.001 (one-way ANOVA with Tukey's post-hoc test).

Suppl. Fig. 5



Supplementary Figure 5. Endothelial PKN2 deficiency results in hypertension. (**A**) Effect of tamoxifen treatment on PKN2 protein expression in mouse lung endothelial cells (MLECs) from WT and Cdh5-CreERT2;Pkn2$^{flox/flox}$ mice. (**B**) Blood pressure in WT (n=8) and Cdh5-CreERT2;Pkn2$^{flox/flox}$ mice (n=8) animals before, during and after induction of tamoxifen. Average blood pressure 3 days before induction was set to 100 %. The bar diagram shows systolic and diastolic arterial blood pressure 4 days before tamoxifen treatment and in the second week after induction. Data represent the mean ± SEM; **P ≤ 0.01 (paired two-tailed Student’s t-test).

Supplementary Table 1. Target sequences of siRNAs used in the siRNA screen (Fig. 2A).

Human		
Target Name	Target Sequence	
PAK2	GAACTGATCATTAACGAGA	Sense
PAK2	TCTCGTTAATGATCAGTTC	antisense
PAK2	GCTGTATGCAGAGAGTGTT	Sense
PAK2	AACACTCTCTGCATACAGC	antisense
PAK2	GTCCTAAAGTTCTACGACT	Sense
PAK2	AGTCGTAGAACTTTAGGAC	antisense
PKD2	CACTCAATTCCGTATCATT	Sense
PKD2	AATGATACGGAATTGAGTG	antisense
PKD2	GCCTTTAAAGCTGATCCGA	Sense
PKD2	TCGGATCAGCTTTAAAGGC	antisense
PKD2	GAAGTTTCATCTTCTATGA	Sense
PKD2	TCATAGAAGATGAAACTTC	antisense
SLK	GAGCAAATGCAGCGTTACA	Sense
SLK	TGTAACGCTGCATTTGCTC	antisense
SLK	GAAGAATTGCGGTTTCTTA	Sense
SLK	TAAGAAACCGCAATTCTTC	antisense
SLK	GATATCAAATTGGCGGATT	Sense
SLK	AATCCGCCAATTTGATATC	antisense
AKT1	CCAAGCACCGCGTGACCAT	Sense
AKT1	ATGGTCACGCGGTGCTTGG	antisense
AKT1	CTCTGCTTTGTCATGGAGT	Sense
AKT1	ACTCCATGACAAAGCAGAG	antisense
AKT1	GGCACTTTCGGCAAGGTGA	Sense
AKT1	TCACCTTGCCGAAAGTGCC	antisense
SNRK	CAGGTATAACCATATCACA	Sense
SNRK	TGTGATATGGTTATACCTG	antisense
SNRK	CAGAAATTCTGCTTGGTGA	Sense
SNRK	TCACCAAGCAGAATTTCTG	antisense

SNRK	GAAACATGAGGAGGGTCTT	Sense
SNRK	AAGACCCTCCTCATGTTTC	antisense
PKN2	CAAATGAGATGTTTGCTAT	Sense
PKN2	ATAGCAAACATCTCATTTG	antisense
PKN2	CAGACTAATGAATTGGCTT	Sense
PKN2	AAGCCAATTCATTAGTCTG	antisense
PKN2	GAGATAGAACAAGCACATT	Sense
PKN2	AATGTGCTTGTTCTATCTC	antisense
MAP2K1	GTCATGGCCAGAAAGCTAA	Sense
MAP2K1	TTAGCTTTCTGGCCATGAC	antisense
MAP2K1	GTCCTACATGTCGCCAGAA	Sense
MAP2K1	TTCTGGCGACATGTAGGAC	antisense
MAP2K1	CAGCGATGGCGAGATCAGT	Sense
MAP2K1	ACTGATCTCGCCATCGCTG	antisense
MAP3K3	GAAACTCAGCTTTATGACA	Sense
MAP3K3	TGTCATAAAGCTGAGTTTC	antisense
MAP3K3	GTCATCAAGGCAACTTGTT	Sense
MAP3K3	AACAAGTTGCCTTGATGAC	antisense
MAP3K3	GATAGAAGCTCAAGCATGA	Sense
MAP3K3	TCATGCTTGAGCTTCTATC	antisense
DAPK3	CACGACATCTTCGAGAACA	Sense
DAPK3	TGTTCTCGAAGATGTCGTG	antisense
DAPK3	GTCTGAAGGAGTACACCAT	Sense
DAPK3	ATGGTGTACTCCTTCAGAC	antisense
DAPK3	CGTTCCTGGGCGAGACCAA	Sense
DAPK3	TTGGTCTCGCCCAGGAACG	antisense
CLK1	GAAAGATTATCATAGTCGA	Sense
CLK1	TCGACTATGATAATCTTTC	antisense
CLK1	CATAGGATGCATTCTTATT	Sense
CLK1	AATAAGAATGCATCCTATG	antisense
CLK1	GATGAACGCACCTTAATAA	Sense
CLK1	TTATTAAGGTGCGTTCATC	antisense
NLK	GTCATTACAGCAATGCTAT	Sense

NLK	ATAGCATTGCTGTAATGAC	antisense
NLK	CCATCATCAGCACTCGCAT	Sense
NLK	ATGCGAGTGCTGATGATGG	antisense
NLK	GAGTTTATACCAGTGACTT	Sense
NLK	AAGTCACTGGTATAAACTC	antisense
AKT3	GAAAGATTGTGTACCGTGA	Sense
AKT3	TCACGGTACACAATCTTTC	antisense
AKT3	CATTTATAATCAGATGTCT	Sense
AKT3	AGACATCTGATTATAAATG	antisense
AKT3	GACATTAAATTTCCTCGAA	Sense
AKT3	TTCGAGGAAATTTAATGTC	antisense
LATS1	GATTACAACTTCACCTATT	Sense
LATS1	AATAGGTGAAGTTGTAATC	antisense
LATS1	CTATTAATGCCAGCATGAA	Sense
LATS1	TTCATGCTGGCATTAATAG	antisense
LATS1	CTACAACAGTCACTGCAAT	Sense
LATS1	ATTGCAGTGACTGTTGTAG	antisense
PKN1	CCTCTGACCCTCGAAGATT	Sense
PKN1	AATCTTCGAGGGTCAGAGG	antisense
PKN1	GGTGACATATCGGTGGAGA	Sense
PKN1	TCTCCACCGATATGTCACC	antisense
PKN1	CCCTTTCCCGCCACGCACT	Sense
PKN1	AGTGCGTGGCGGGAAAGGG	antisense
MAP3K7	GTAGATCCATCCAAGACTT	Sense
MAP3K7	AAGTCTTGGATGGATCTAC	antisense
MAP3K7	CATGCAACCCAAAGCGCTA	Sense
MAP3K7	TAGCGCTTTGGGTTGCATG	antisense
MAP3K7	GCCATTACAGTATCCTTGT	Sense
MAP3K7	ACAAGGATACTGTAATGGC	antisense
GSK3B	GACACTATAGTCGAGCCAA	Sense
GSK3B	TTGGCTCGACTATAGTGTC	antisense
GSK3B	CAATGTTTCGTATATCTGT	Sense
GSK3B	ACAGATATACGAAACATTG	antisense

GSK3B	CACAGAATTTAAATTCCCT	Sense
GSK3B	AGGGAATTTAAATTCTGTG	antisense
ROCK1	GTAACCAAAGCTCGTTTAA	Sense
ROCK1	TTAAACGAGCTTTGGTTAC	antisense
ROCK1	GTGATTGGTAGAGGTGCAT	Sense
ROCK1	ATGCACCTCTACCAATCAC	antisense
ROCK1	CATGCAAGCGCAATTGGTA	Sense
ROCK1	TACCAATTGCGCTTGCATG	antisense
PIM3	GGGCGTGCTTCTCTACGAT	Sense
PIM3	ATCGTAGAGAAGCACGCCC	antisense
PIM3	CGGTTCGGGTGCGCTGCTC	Sense
PIM3	GAGCAGCGCACCCGAACCG	antisense
PIM3	GTCGCTGGGCGTGCTTCTC	Sense
PIM3	GAGAAGCACGCCCAGCGAC	antisense
HIPK1	GCAATCAGCTCAATACAGT	Sense
HIPK1	ACTGTATTGAGCTGATTGC	antisense
HIPK1	CAAATACAAGCACAAATCT	Sense
HIPK1	AGATTTGTGCTTGTATTTG	antisense
HIPK1	CATTTGGACAGGTGGCTAA	Sense
HIPK1	TTAGCCACCTGTCCAAATG	antisense
TRIO	CTACTCAGCTGATATTGGA	Sense
TRIO	TCCAATATCAGCTGAGTAG	antisense
TRIO	GCAAGTTAGAGAACGGTTA	Sense
TRIO	TAACCGTTCTCTAACTTGC	antisense
TRIO	CAGAAACAGAGGACTAATT	Sense
TRIO	AATTAGTCCTCTGTTTCTG	antisense
CDK9	GCTGCTAATGTGCTTATCA	Sense
CDK9	TGATAAGCACATTAGCAGC	antisense
CDK9	GCCAGAAGCGGAAGGTGAA	Sense
CDK9	TTCACCTTCCGCTTCTGGC	antisense
CDK9	GGCCAAACGTGGACAACTA	Sense
CDK9	TAGTTGTCCACGTTTGGCC	antisense
MAP2K3	CTCATGGACCTGGACATCA	Sense

MAP2K3	TGATGTCCAGGTCCATGAG	antisense
MAP2K3	GAGACGTGTGGATCTGCAT	Sense
MAP2K3	ATGCAGATCCACACGTCTC	antisense
MAP2K3	CACAGCAAGCTGTCGGTGA	Sense
MAP2K3	TCACCGACAGCTTGCTGTG	antisense
PKN3	CAGGAACGCATCTTCTCTA	Sense
PKN3	TAGAGAAGATGCGTTCCTG	antisense
PKN3	CCTGAAGTTGGATAACCTT	Sense
PKN3	AAGGTTATCCAACTTCAGG	antisense
PKN3	CTTCTACGTGGCTTGTGTT	Sense
PKN3	AACACAAGCCACGTAGAAG	antisense
Piezo1	GTGTTTGGTCTCAAGGACT	Sense
Piezo1	GTGTCTACTTCCTGCTCTT	antisense

Bovine		
Target Name	Target Sequence	
PKN2	GTCCAAACCAGCAGCACTA	Sense
PKN2	TAGTGCTGCTGGTTTGGAC	antisense
PKN2	CTGGAAATTTCAGTTTATT	Sense
PKN2	AATAAACTGAAATTTCCAG	antisense
PKN2	GCAAGATTTAATGAATCAA	Sense
PKN2	TTGATTCATTAAATCTTGC	antisense

Supplementary Table 2. Primer sequences of qRT-PCR (Fig. 2A).

Oligo Name	Sequence 5' to 3'	
SNRK	tcttgcatattccgctcca	Forward
SNRK	ggatcactcccagactccaa	Reverse
PAK2	aaaaaggggttcagccaaag	Forward
PAK2	agataacggtttggccagttt	Reverse
TRIO	agtccacccagagcaacg	Forward
TRIO	cgtgtaatcgtgtgtcaccaa	Reverse
AKT3	ttgctttcagggctcttgat	Forward
AKT3	cataatttcttttgcatcatctgg	Reverse
ROCK1	gatcccaaatcggaagtgaa	Forward
ROCK1	caaatcatataccaaagcatccaa	Reverse
CLK1	tgtggggaaacgttgtctg	Forward
CLK1	tgctatgtgatctcttccttctttt	Reverse
MAP3K3	ggatgcctggatatgagacc	Forward
MAP3K3	tgtgctcgaacttgattctga	Reverse
SLK	ggatgccaggtggactacat	Forward
SLK	ggatgggtttgttggaatca	Reverse
HIPK1	ggcgctggttgaacagac	Forward
HIPK1	agctaccccaggcaactgt	Reverse
AKT1	ggctattgtgaaggagggttg	Forward
AKT1	tccttgtagccaatgaaggtg	Reverse
GSK3B	gaaagtattgcaggacaagagattt	Forward
GSK3B	cggactatgttacagtgatctagctt	Reverse
CDK9	ttcggggaggtgttcaag	Forward
CDK9	atctcccgcaaggctgtaat	Reverse
DAPK3	gacgagggagggagagaatc	Forward
DAPK3	agcctggagataggacctcag	Reverse
MAP3K7	agctttatggagcctgcttg	Forward
MAP3K7	agagccccttcagcatatt	Reverse
PIM3	ccacctcccggtgaagat	Forward
PIM3	ccacctggtacgccttctc	Reverse
PKD2	caagaaaaatgtctggctgga	Forward

PKD2	ttgctgggaattcaaccaat	Reverse
MAP2K3	gaagggctacaatgtcaagtcc	Forward
MAP2K3	taagggaaccgcaggatg	Reverse
NLK	aagatgcccaacgtcttcc	Forward
NLK	ggagtatgtcaagggcagaga	Reverse
MAP2K1	ttttaggaaaagttagcattgctgt	Forward
MAP2K1	agggcttgacatctctgtgc	Reverse
LATS1	aaatgagttaccaagatcctcgac	Forward
LATS1	cggttaactgattgctgcac	Reverse
PKN3	ccgggacgagatagagagc	Forward
PKN3	aaggagggagagcaggaaag	Reverse
GAPDH	gcatcctgggctacactga	Forward
GAPDH	ccagcgtcaaaggtggag	Reverse