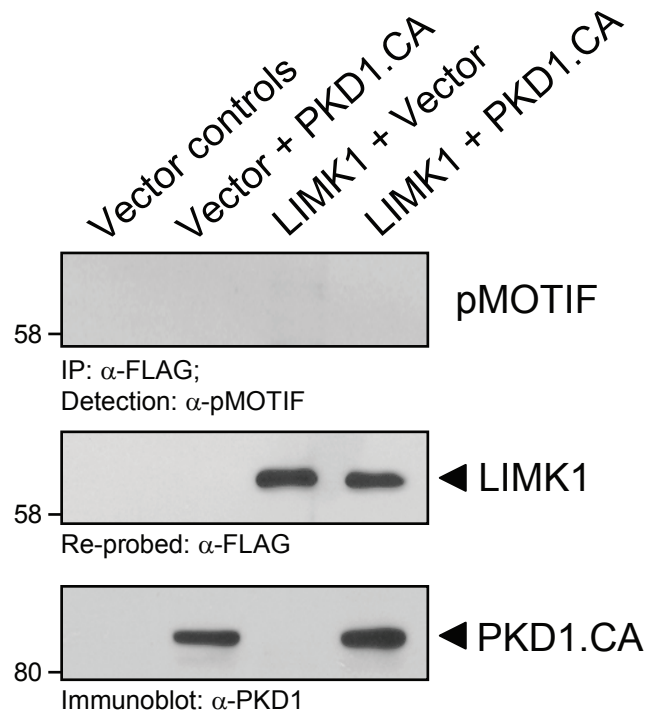
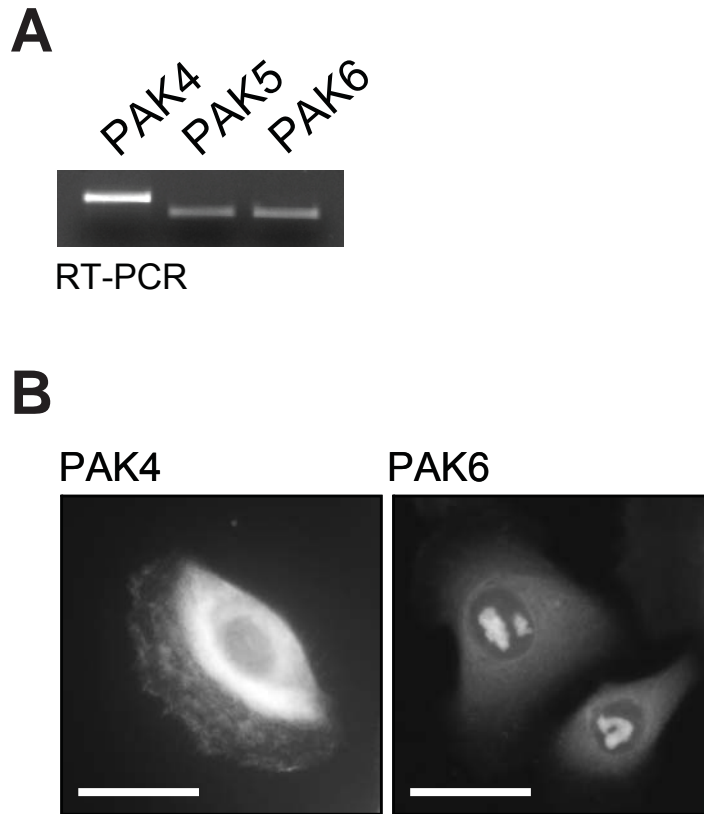


Supplemental Figure 1



Suppl. Fig. 1: **LIMK is not a PKD target.** HeLa cells ( $0.65 \times 10^6$  cells, 6 cm dish) were transfected with vector controls, FLAG-tagged LIMK1 or constitutively-active PKD1 as indicated. LIMK1 was immunoprecipitated ( $\alpha$ -FLAG) and analyzed for PKD-mediated phosphorylations using the pMOTIF antibody that is specific for phosphorylated PKD substrates. Samples were stripped and re-probed for LIMK1 using  $\alpha$ -FLAG antibodies. Lysates were analyzed by Western blot for PKD1.CA expression.



Suppl. Fig. 2: **Expression and localization of PAK4 and PAK6.** **A:** RNA extraction from HuMEC cells followed by RT-PCR using primers against PAK4, 5 and 6. Cellular mRNA isolation was performed using RNA-Bee from TEL-TEST (Friendswood, TX) according to the manufacturers instructions and was transcribed into cDNA using the following transcription reaction; 1  $\mu$ l oligo dT primer and 2 $\mu$ l RNA were incubated in a total of 2  $\mu$ l water at 70 °C for 5 minutes. Then, 5X buffer, 1  $\mu$ l RNAsin, 1  $\mu$ l dNTP mix, 2.4  $\mu$ l  $MgCl_2$  and 1 $\mu$ l reverse transcriptase, all from Promega (Madison, WI) were added to a total volume of 20  $\mu$ l. The resulting cDNA pool was subjected to PCR analysis using specific primer sets. Primer pairs used were: 5'-ACACCAGGATGAACGAGGAG-3' and 5'-TTGTGCAGGTTCTTCAGTCG-3' for a 394 bp fragment of PAK4, 5'-TGCTTTTCGGTAGAGCTGGAT-3' and 5'-TTGTATGCCACTGAGGTGGA-3' for a 302 bp fragment of PAK5, 5'-TTGTATGCCACTGAGGTGGA-3' and 5'-TGCTTTTCGGTAGAGCTGGAT-3' for a 302 bp fragment of PAK6. **B:** HuMEC cells ( $1 \times 10^4$  cells, ibiTreat  $\mu$ -slide) were subjected to indirect immunofluorescence analysis. Samples were indirectly labelled with  $\alpha$ -PAK4 (Abcam ab62509, dilution: 1:200) or  $\alpha$ -PAK6 (Abcam ab62511, dilution: 1:200) and secondary Alexa Fluor 546 anti-rabbit antibodies (dilution: 1:800).