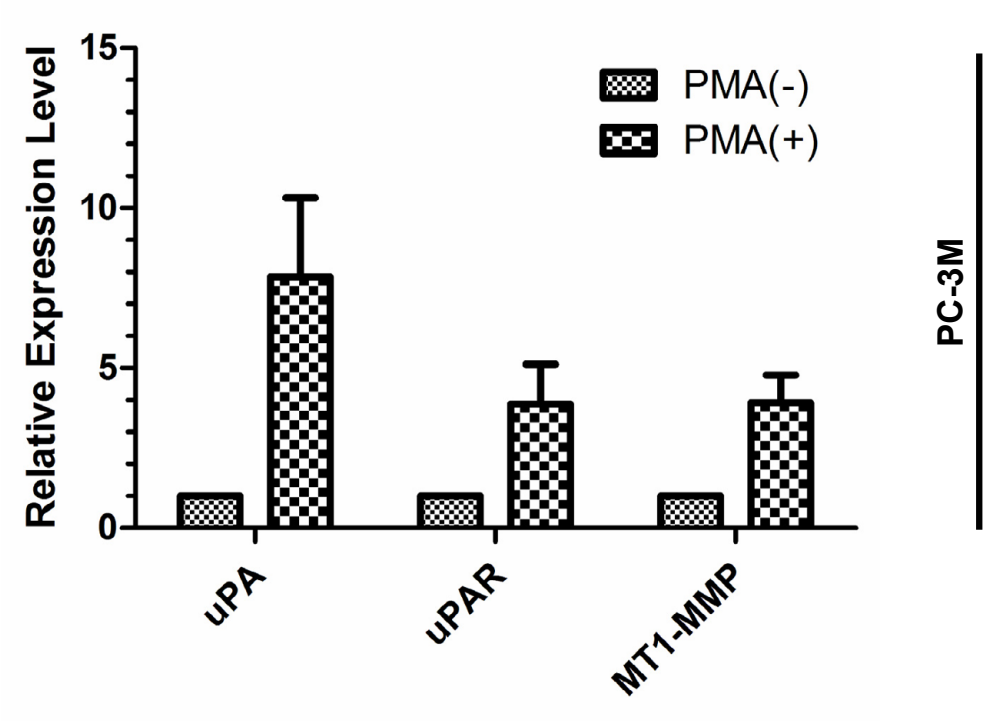

Fig. S1. PMA was able to induce uPA, uPAR and MT1-MMP expression at mRNA level. PC-3M cells was serum starved for 24h and further treated with 100 nM PMA for 24h, followed by analysis with RT-QPCR for mRNA levels of the indicated genes. Data represent the mean ± s.e.m of 3 independent experiments.

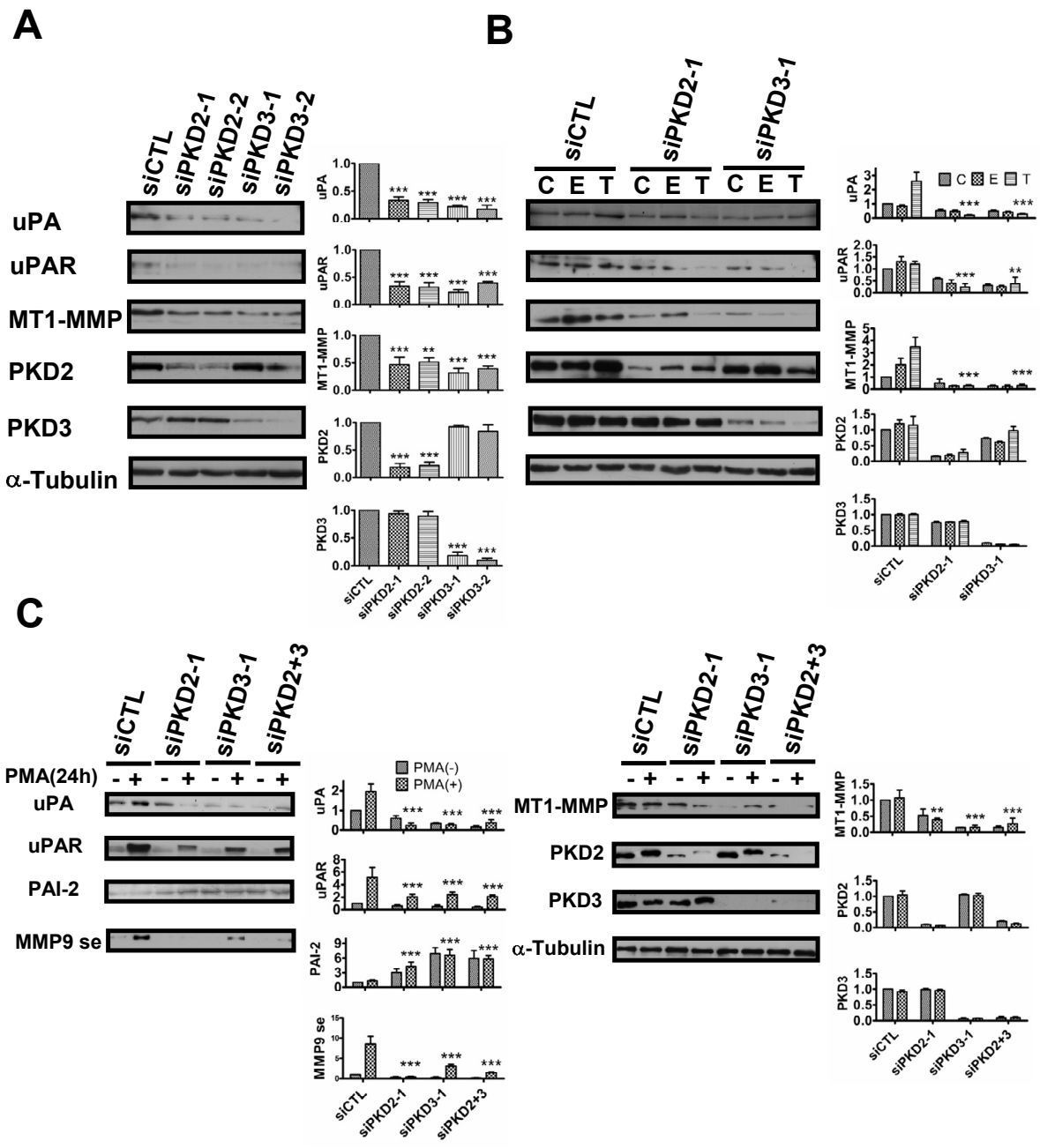

Fig. S2. PKD2 or PKD3 is critical for expression of invasion/metastasis genes of uPA/uPAR and MMP pathways in DU145 cells. (A) DU145 cells were transfected with control siRNA (siCTL), PKD2 siRNA (siPKD2-1 or siPKD2-2), or PKD3 siRNA (siPKD3-1 or siPKD3-2). After cultured in medium containing 10% FBS for 24h, cells were subjected to transwell analysis as indicated in Fig. 1D, with remaining cells being seeded back into the plate. After additional 24h, whole cell lysates from remaining cells were separated by SDS-PAGE and immunoblotted with antibodies specified. (B) DU145 cells were transfected as indicated. Twenty-four hours after transfection, cells were serum-starved for further 24h and treated with medium (C), 50ng/mL EGF (E) or 10ng/mL TNF-α (T) for 24h. Whole cell lysates were separated by SDS-PAGE and immunoblotted with antibodies specified. (C)DU145 cells were transfected and starved as above, followed by treating with medium [PMA(-)] or 100nM PMA [PMA(+)] for further 24h. α-tubulin was used as a internal loading control and PKD2 and PKD3 were detected to ensure their sufficient knockdown. MMP9 se: secreted MMP9. Quantitation of each blot was indicated right to the blots. The statistical data of the target protein/α-Tubulin was shown and this value in the siCTL was set to 1, with all other groups normalized to this group. Data represent the mean ± s.e.m. of 3 independent experiments. Data in (A) were analyzed by one-way ANOVA, followed by he Dunnett post hoc test for significance against siCTL. **, $p < 0.01$ and ***, $p < 0.001$ versus siCTL. Quantitative data in (B) and (C) were analyzed by 2-way ANOVA with multiple comparisons, followed by the Bonferroni post hoc test for significance. *, $p < 0.05$ **, $p < 0.01$, and ***, $p<0.001$ versus siCTL+PMA or siCTL+T.

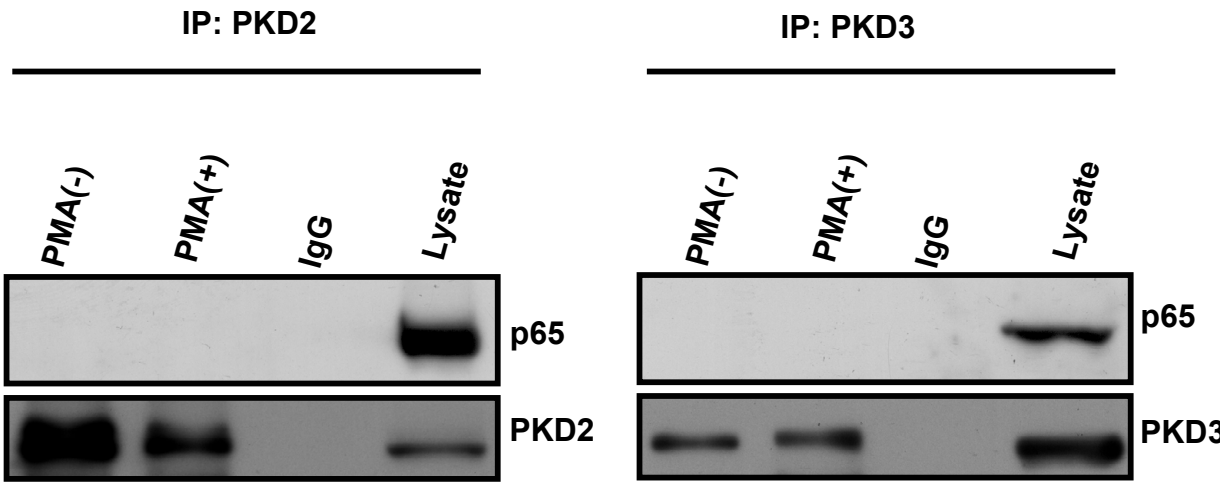

Fig. S3. PKD2 and PKD3 did not interact with p65 in PC-3M cells. PC-3M cells were grown to 70%-80% confluent and serum-starved for 24h, then stimulated with 100nM PMA for 15 min. Whole cell lysates were immunoprecipitated with antibody against endogenous PKD2/3 and co-precipitating p65 NF-κB were detected by immunoblotting.

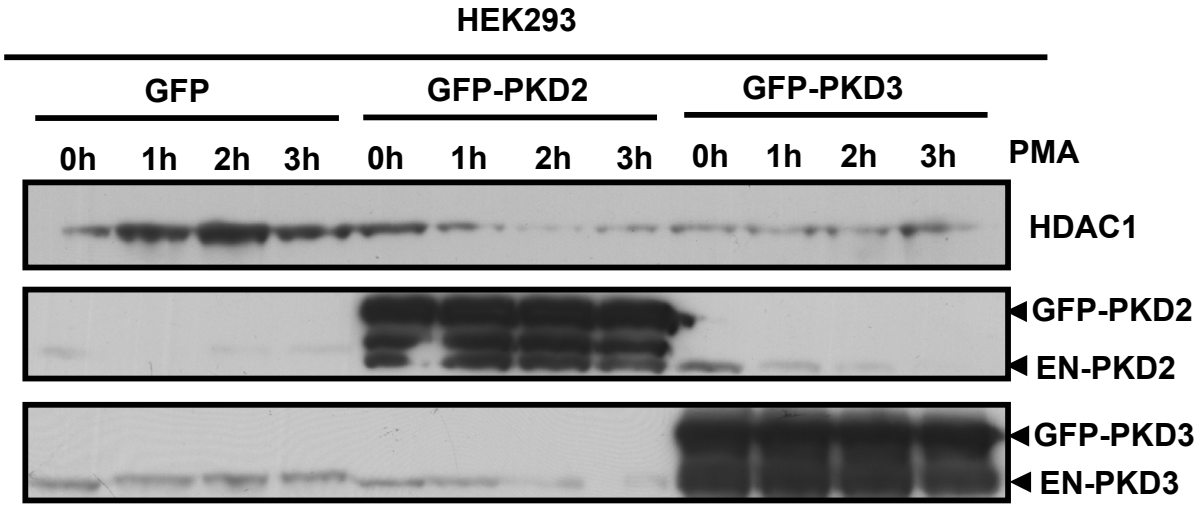

Fig. S4. PKD2 and PKD3 suppressed HDAC1 expression in HEK293 cells. HEK293 cells were transfected with pEGFP-C2, pEGFP-PKD2 or pEGFP-PKD3. After starvation for 24h, cells were treated with 100 nM PMA for indicated time course. Expression level of HDAC1 in the whole cell lysates was revealed by western blotting using a antibody against HDAC1. PKD2 and PKD3 were also detected to ensure essential over-expression of these 2 kinases.

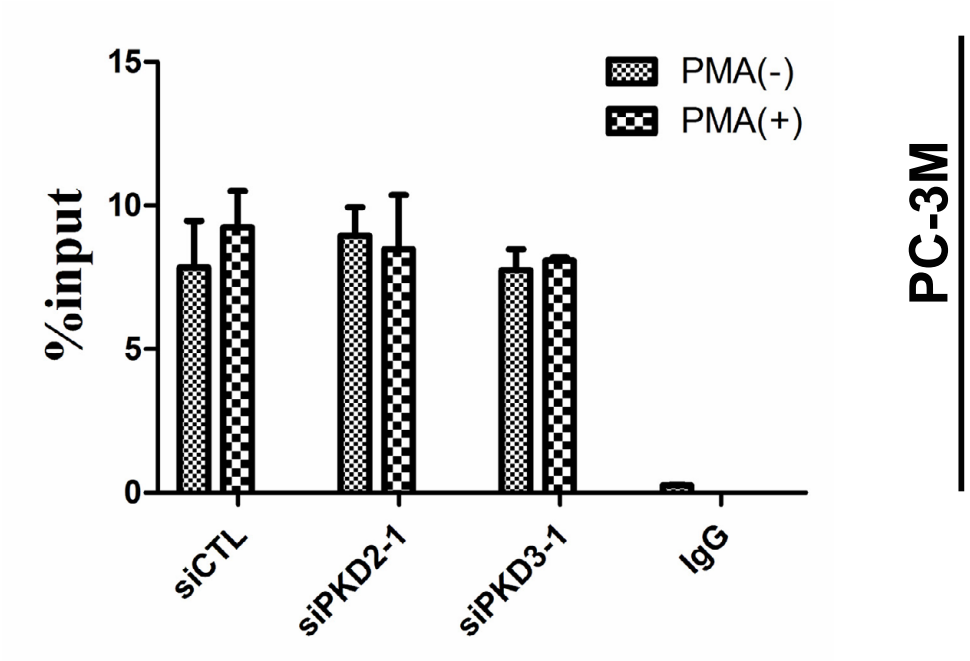

Fig. S5 Acetylation of histone H3 Lys9/Lys14 at the uPA promoter remained unchanged after PKD2 and PKD3 depletion. PC-3M cells were transfected with control siRNA (siCTL), PKD2 siRNA (siPKD2-1), PKD3 siRNA (siPKD3-1) or both (siPKD2+3). Twenty-four hours after transfection, cells were serum-starved for additional twenty-four hours and treated with PMA for 1 hour. Whole cell lysates were immunoprecipitated with a antibody raised against histone H3 acetylated at Lys9/Lys14, the co-precipitaing chromosome fragment in vivo were amplified using primers against uPA promoter and quantified by realtime PCR. Results are presented as a ratio of immunoprecipitated product to the input product. Data represent the mean ± s.e.m of 2 independent experiments.

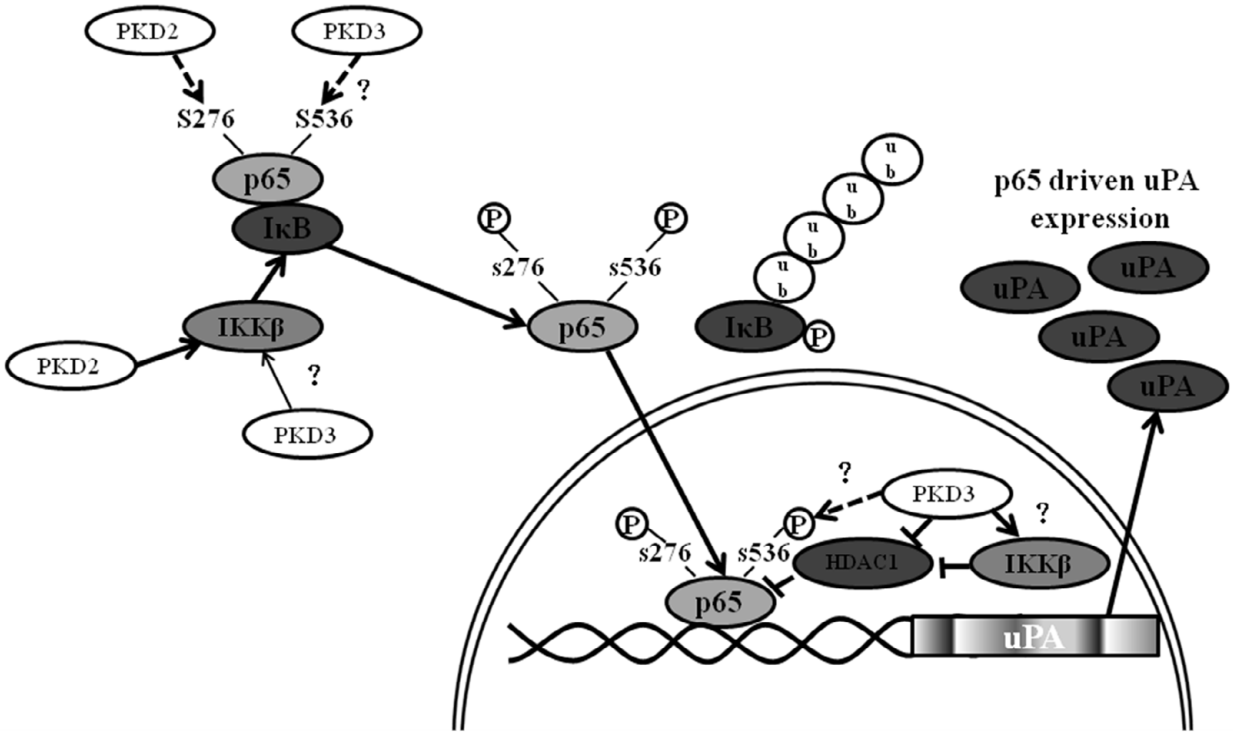

Fig. S6. Schematic model of the cooperative role of PKD2 and PKD3 in the expression of invasion genes such as uPA. On one hand, PKD2 primarily mediates IKK phosphorylation, leads to IkB phosphorylation and subsequent degradation, and directs p65 nuclear translocation and DNA binding. In addition, while PKD2 mediates S276 phosphorylation, PKD3 is primarily responsible for S536 phosphorylation, both of which contribute to the transactivity of p65. On the other hand, PKD3 mediates HDAC1 depletion, clearing it out of the p65-binded uPA promoter region and makes this region accessible to transcriptional machinery. Doted arrow indicates the regulation might not be direct, whereas a question mark indicates where PKD3 mediates S536 phosphorylation (within cytosol or nucleus) remains unclear. Also, where PKD3 interact with IKKβ (within cytosol or nucleus) needs further investigation.