Legends for supplementary figures

Supplement Fig. S1. Absence of PKCβ1 interaction with pErk1/2 in the HDF cells treated with TPA. (A) Immunocytochemical findings of HDF old cells treated with TPA (50 ng/ml) or DMSO (0.01%) for 30 min by using anti-PKCα (A) and anti-PKCβ1 (B) antibodies. Note nuclear translocation of PKCα, but not PKCβ1, after TPA treatment. (C) Immunoblot analysis of nuclear (Nu) and cytoplasmic (Cyt) fractions of HDF senescent cells treated with TPA for 30 min. Note nuclear translocation of pErk1/2 alone, but not with PKCβ1, upon TPA treatment. (D) Reciprocal immunoprecipitation of HDF old cell lysates with anti-pErk1/2 and anti-PKCβ1 antibodies. Note absence of PKCβ1 interaction with pErk1/2 in the HDF old cells.

Supplement Fig. S2. Changes of pErk1/2 and PKCα localization in HDF cells after TPA treatment. (A) Immunofluorescence findings. TPA treatment significantly increased pErk1/2 and PKCα fluorescence in the nuclei of senescent cells in 30 min, and the fluorescence of PKCα in nuclei was decreased more rapidly than that of the pErk1/2. (B) Time dependent change of the nuclear pErk1/2 in response to TPA. The cells with pErk1/2 in nuclei were counted and the percentages of the cells based on total cell numbers were presented. Note significant induction of nuclear localization of pErk1/2 upon TPA treatment for 1 h and its maintenance until 8 h. However, the level was significantly reduced at 8 h, compared with that of the 4 h after TPA treatment. (C) Immunoblot analysis confirming rapid loss of PKCα expression than that of pErk1/2 after TPA treatment. As shown above in Fig S2A, loss of PKCα expression was faster than that of the pErk1/2 in 4 h of TPA treatment in the HDF old cells.

Supplement Fig. S3. Failure of pErk1/2 translocation to nuclei by mutant PKCα without regulatory domain despite its catalysis. (A) Diagrams showing the mutant PKCα constructs with potential MAPK docking motifs in the regulatory and catalytic domains of PKCα constructs. MAPK docking motifs on PKC molecule was predicted based on the eukaryotic linear motif resources from website http://elm.eu.org and the references were described (Bardwell et al., 2001; Debata et al., 2010; Yang et al., 1998). Wild-type PKCα (wt-PKCα), double mutation in the regulatory domain of PKCα at $R^{159,161}G$ residues [mt-PKCα($R^{159,161}G$)], kinase dead PKCα with $K^{368}R$ mutation (KD-PKCα), and constitutively active PKCα with deletion of 1–325 residues (CA-PKCα). **(B)** Immunocytochemistry showing localizations of PKCα and pErk1/2 in Huh7 cells transfected with either wt-PKCα or mutant PKCα constructs. Huh7 cells were transiently transfected for 48 h with wt-PKCα, CA-PKCα or KD-PKCα cloned in the control vector pcDNA3-HA, and the cells were then treated with either DMSO (0.01%) or TPA for 30 min before subjected to immunocytochemistry. Co-localization of wt-PKCα (green) and pErk1/2 (red) in nucleus was clearly observed after TPA treatment only in the wt-PKCα transfected cells, but not in any mutant. ICC showed activated form of the wild type-PKCα and the KD-PKCα in response to TPA, as well as the CA-PKCα, whereas nuclear translocation of pErk1/2 was failed in the cells with CA-PKCα and KD-PKCα expressers, suggesting the requirement of kinase activity and regulatory domain of PKCα to carry Erk1/2 into nuclei of the cells.

Supplement Fig. S4. Relative values of the differentially expressed genes (DEG) in the HDF old cells treated with TPA for 8 h and 24 h based on the DMSO treated control. HDF old cells were incubated with TPA (50 ng/ml) for 8 h and 24 h,

and the RNAs isolated from the cells were analyzed by RNA sequencing. Relative values of the differentially expressed genes in the TPA treated cells based on the DMSO control were employed to heatmap preparation. (**A**) **Heatmap and hierarchical clustering of the common 222 differentially expressed genes.** Heatmap was generated by hierarchical clustering of up- and down-regulated DEG values of 222 genes in the TPA 8 h and the TPA 24h based on the DMSO control in the HDF old cells. The DEGs between the two selected biological conditions were analyzed by Cuffdiff software in Cufflinks package with the significant thresholds of p-value < 0.001 or false discovery rate < 0.05 after multiple testing corrections. (**B**) **Heatmap and hierarchical clustering of 53 differentially expressed genes.** Heatmap generated by hierarchical clustering of DEG values of 53 genes revealed significant changes in the TPA 8 h and the TPA 24 h treated HDF old cells compared with the DMSO control with the significance threshold as false discovery rate and p-value < 0.05. Functional category analysis with DEGs was performed by DAVID (http://david.abcc.ncifcrf.gov), Enrichr (http://amp.pharm.mssm.edu/Enrichr/) or PANTHER (http://pantherdb.org/) methods.

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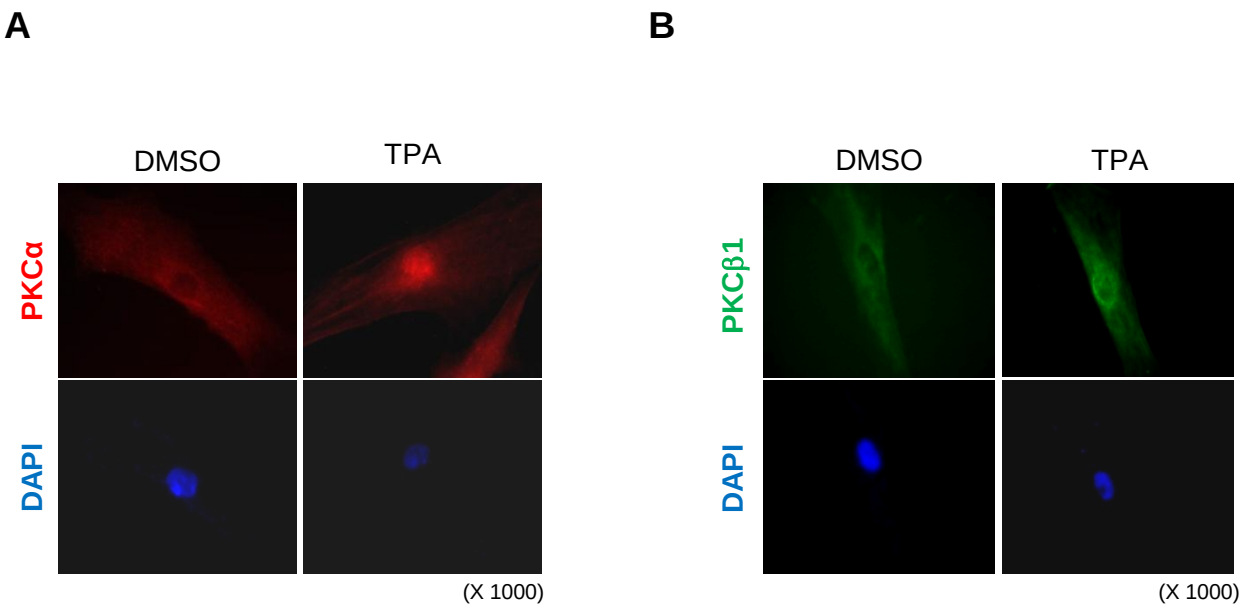
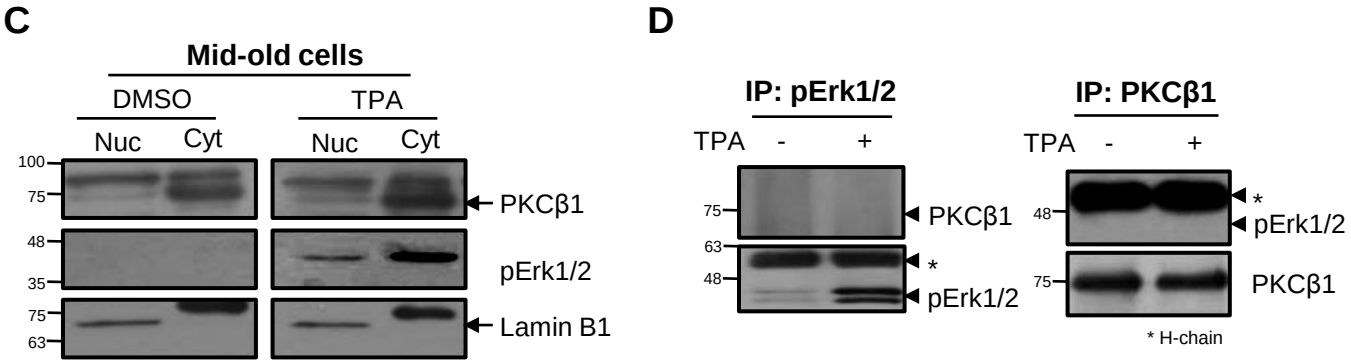
Supplement Fig. 1





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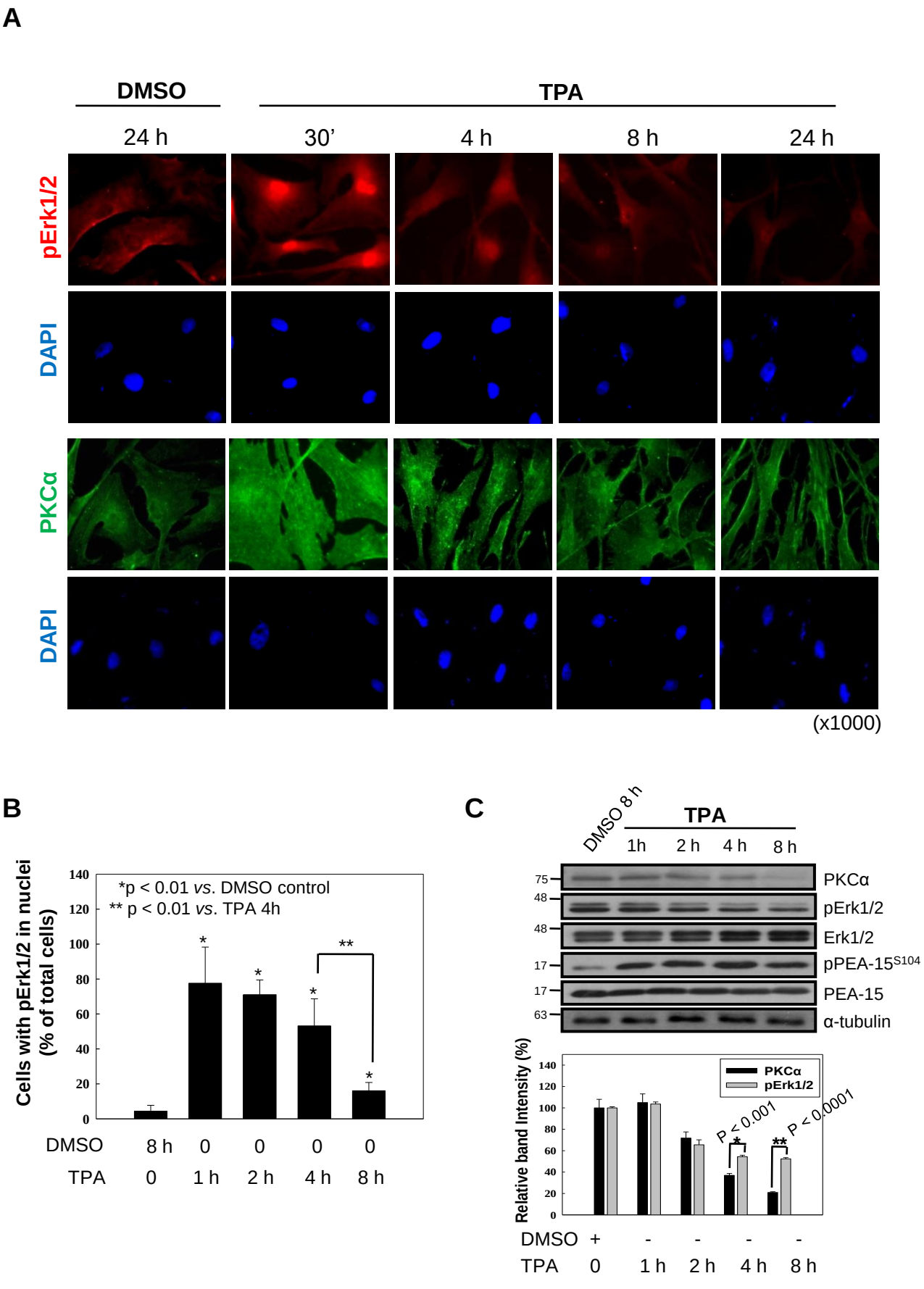

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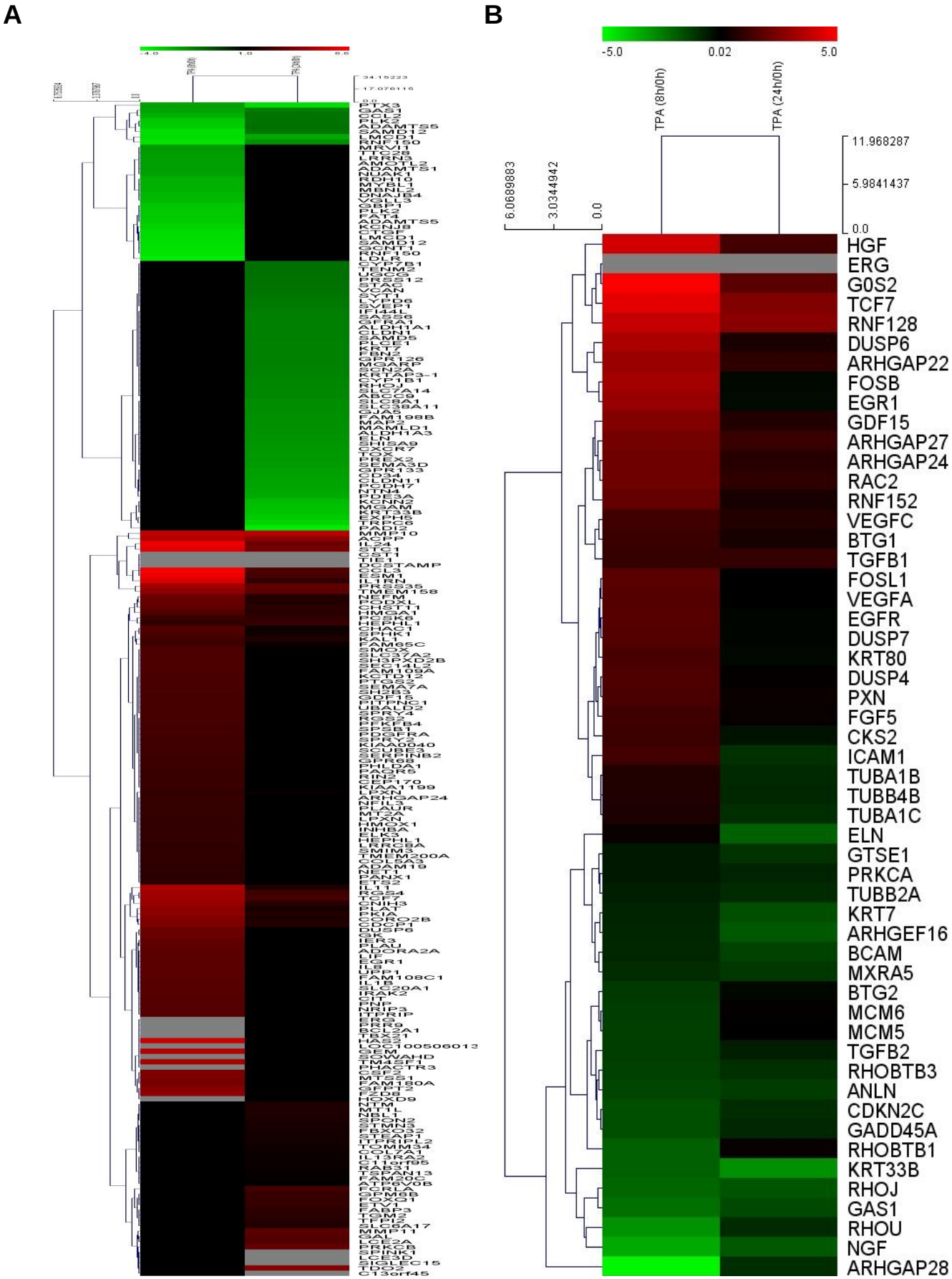


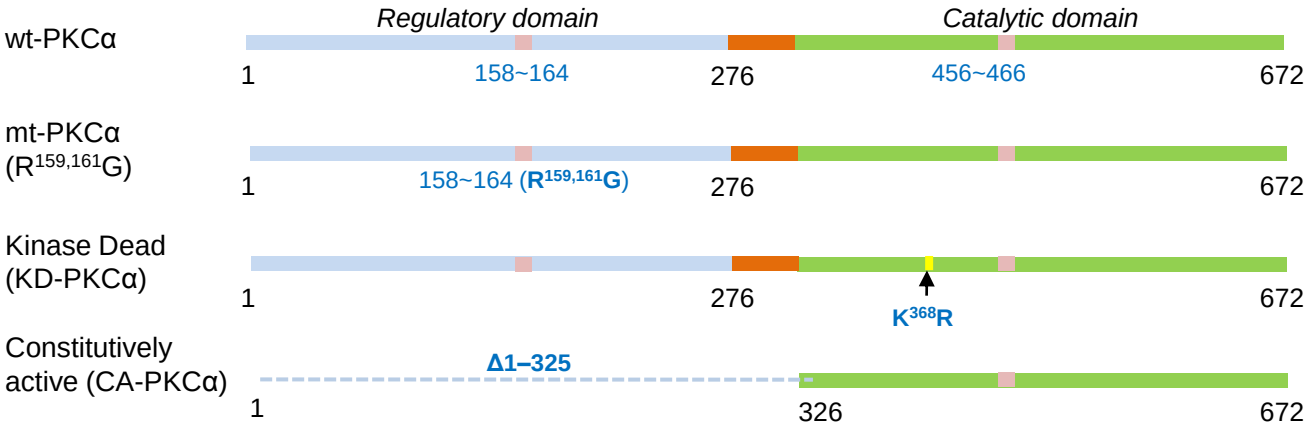
Supplement Fig. 3

A

Predicted potential MAPK docking motifs in PKCα molecule

158~164 : KRGRIYL

456~466 : KRGIIYRDLKL



B