Supplement Figure S1

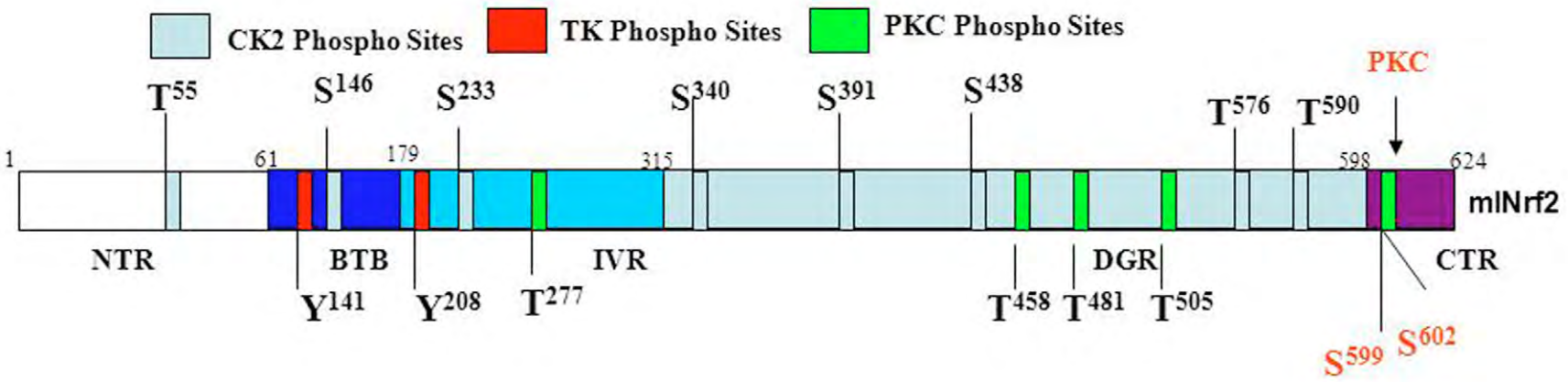

Figure S1. Systematic presentation of INrf2 different domains. Prosite search predicated serine, threonine and tyrosine phosphorylation sites of INrf2 are shown. Predicted kinases are also presented. C-terminal unique PKC site which is responsible for INrf2S602 and INrf2S599 phosphorylation is shown. CK2- Casein Kinase 2; TK-Tyrosine Kinase; PKC-Protein Kinase C.

Supplement Figure S2

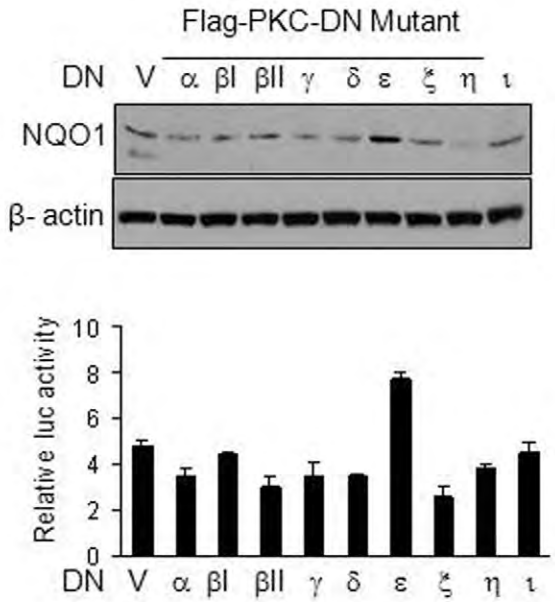

Figure S2. Effect of overexpression of various PKC-DNs mutants on Nrf2 downstream NQO1 expression. Upper panel, immunoblotting of HepG2 cell lysates after transfection of PKC-DNs with NQO1 and actin antibodies; Lower panel, NQO1-ARE luciferase activity. The data shown are mean ± S.D. of three independent transfection experiments. V- Vector.

Supplement Figure S3

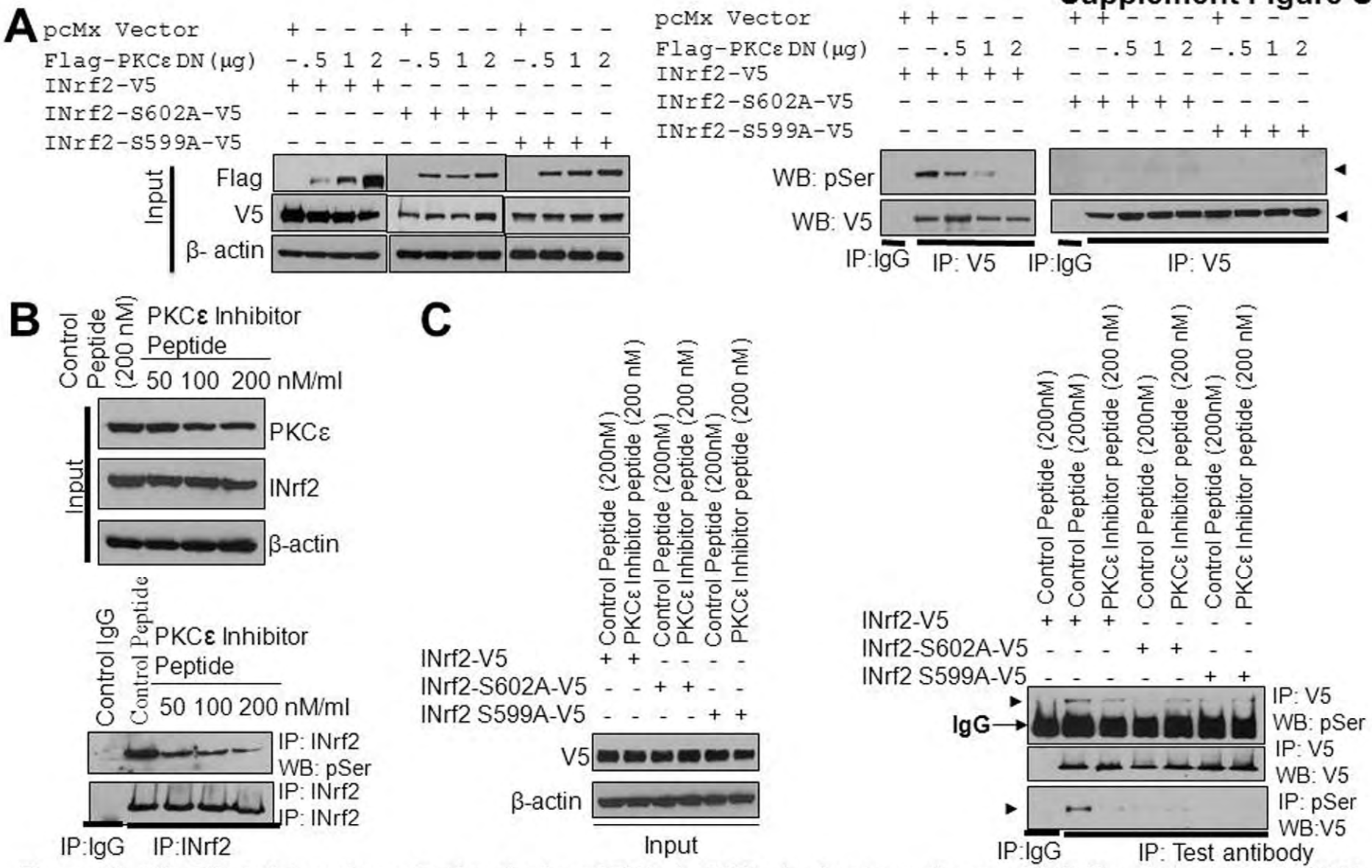

Figure S3. (A) Effect of dose dependent inactivation of PKCε by PKCε dominant negative on wild-INrf2 and mutant INrf2 S602A or INrf2S599A serine phosphorylation. The serine phosphorylation was analyzed by immunoprecipitation and immunoblotting using specific antibodies (left and right panels). (B) Effect of BSA control peptide and PKCε inhibitor peptide on endogenous INrf2 serine phosphorylation (upper and lower panels). (C) Effect of BSA control peptide or PKCε inhibitor peptide on transfected wild INrf2-V5, and INrf2S602A-V5 or INrf2S599A-V5 mutant serine phosphorylation (left and right panels). The serine phosphorylation of INrf2 and mutant INrf2 was analyzed by immunoprecipitation and immunoblotting using specific antibodies.

Supplement Figure S4

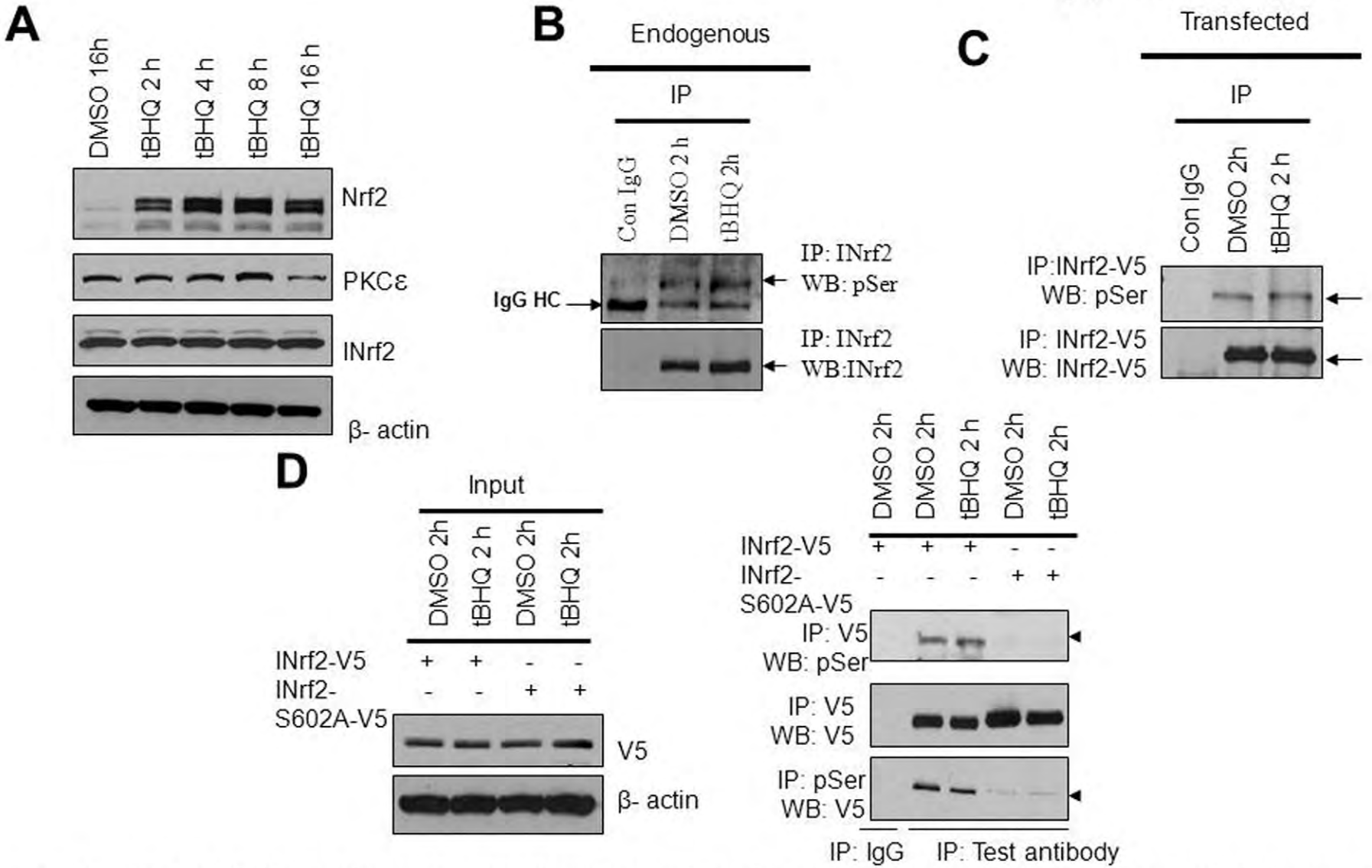

Figure S4. Effect of tBHQ on INrf2 serine phosphorylation. (A) HepG2 cells were treated with DMSO or tBHQ for indicated times and lysates were immunoblotted with specific antibodies. (B) DMSO or tBHQ treated (2h) cell lysates were subjected for INrf2 immunoprecipitation followed by immunoblotting with phosphoserine antibodies. (C) HepG2 cells were transfected with INrf2-V5 and treated with DMSO or tBHQ for (2h) and lysates were immunoprecipitated with anti V5 antibody and immunoblotted with phosphoserine antibody. (D) HepG2 cells were transfected with INrf2-V5 or mutant INrf2S602A plasmids and treated with DMSO or tBHQ for (2h). Cell lysates (1mg) were immunoprecipitated and immunoblotted as indicated (left and right panels).

Supplement Figure S5

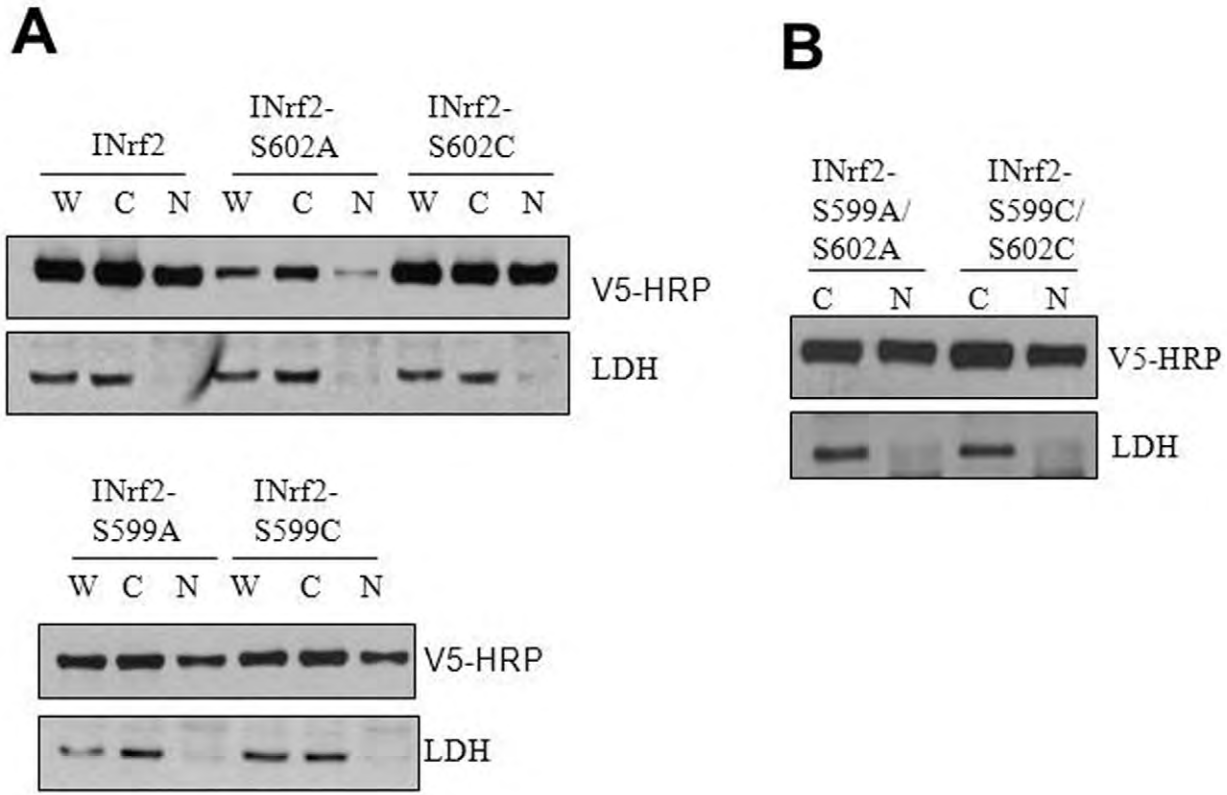

Figure S5. (A&B) Cytosolic and nuclear localization of INrf2-V5 and various c-terminal serine single and double mutants of INrf2. HepG2 cell were transfected with wild type INrf2-V5 and various INrf2 mutant constructs as indicated and whole, cytosolic and nuclear lysates were immunoblotted. W- whole. C- cytosol. N-nucleus.

Supplement Figure S6

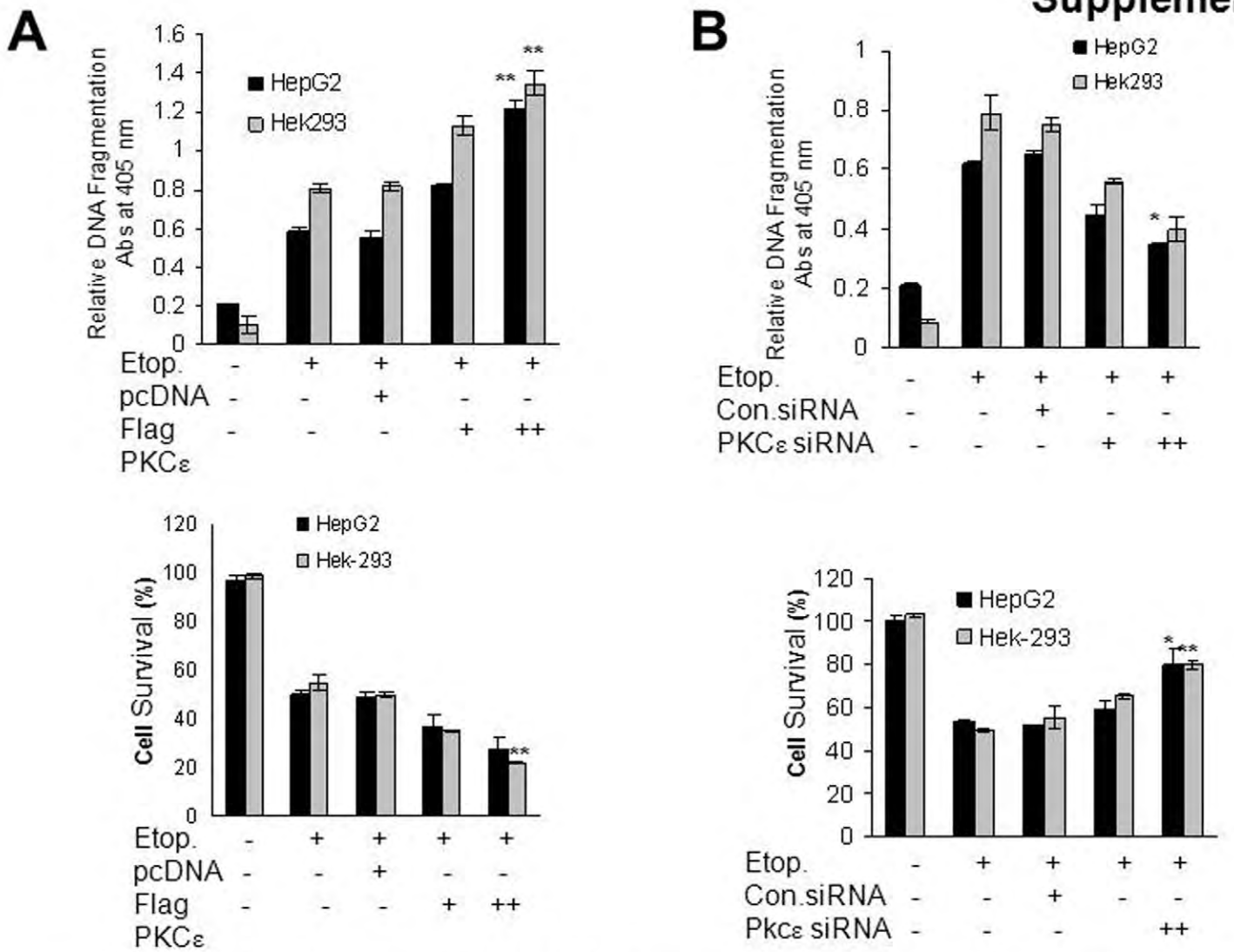

Figure S6. (A) Effect of overexpression of PKCε on etoposide mediated DNA fragmentation and cell survival in HepG2 and Hek293 cell were measured (upper and lower panels). (B) Effect of control siRNA (100 nM) and PKCε siRNA (50 and 100 nM) on etoposide mediated DNA fragmentation and cell survival in HepG2 and Hek293 cell were measured (upper and lower panels). Small histone associated DNA fragmentation was measured by cell death kit (Roche) and cell survival by MTT assay. The experiments repeated thrice and the data shown are mean ± S.D. of three independent experiments. Etop.- etoposide.

Supplement Figure S7

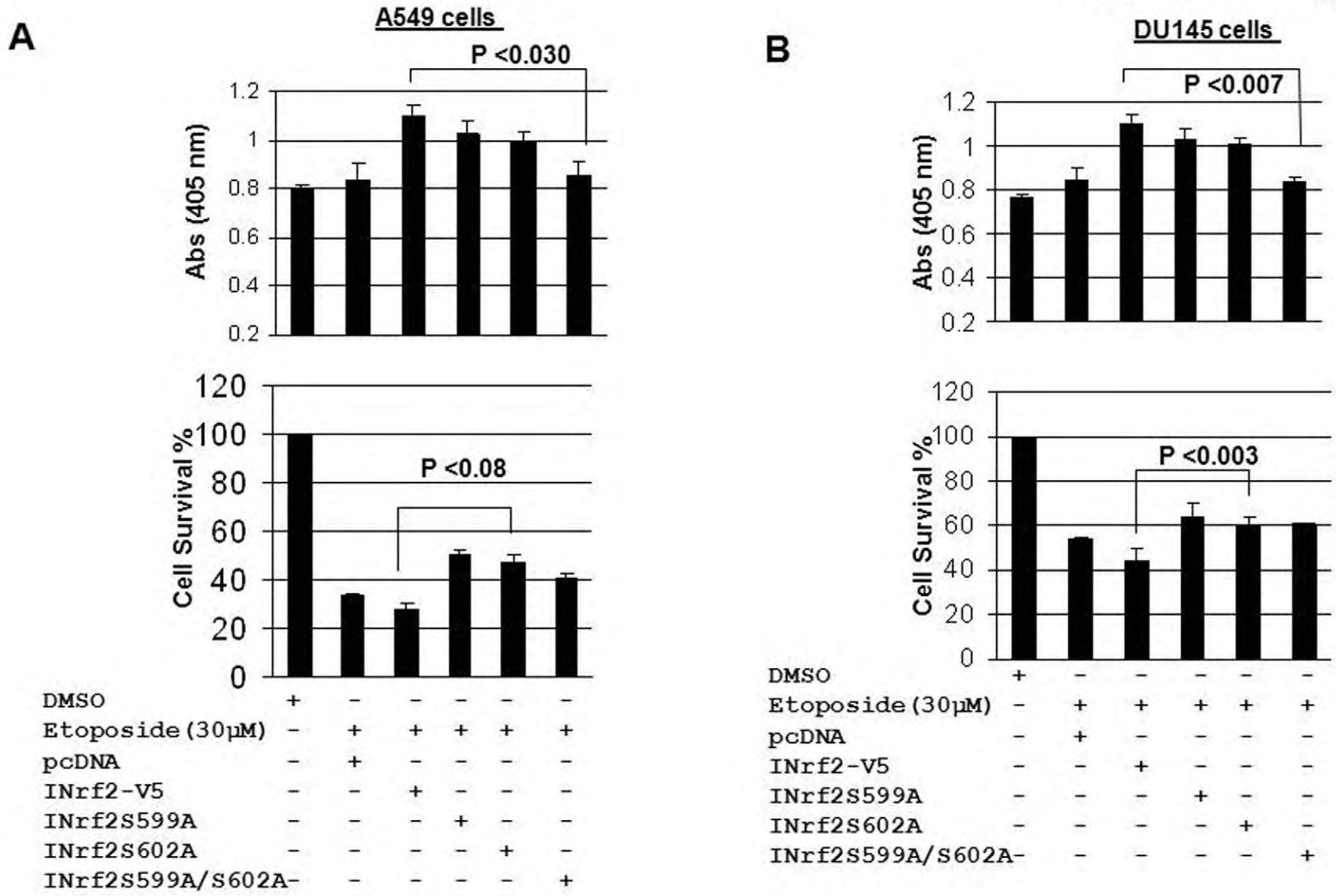

Figure S7. Effect of wild type INrf2-V5, INrf2S599A-V5, INrf2S602A-V5 and INrf2S599A/S602A-V5 double mutant expression in A549 cells and DU145 cells and etoposide treatments on cell death/survival. A&B) Lung cancer A549 and Prostate cancer DU145 cells were transfected with indicated plasmids for 18h and treated with etoposide for additional 36h. Small histone associated DNA fragmentation was measured by cell death kit (Roche) and cell survival by MTT assay. The data shown are mean ± S.D. of three independent treatments.

Supplement Figure S8

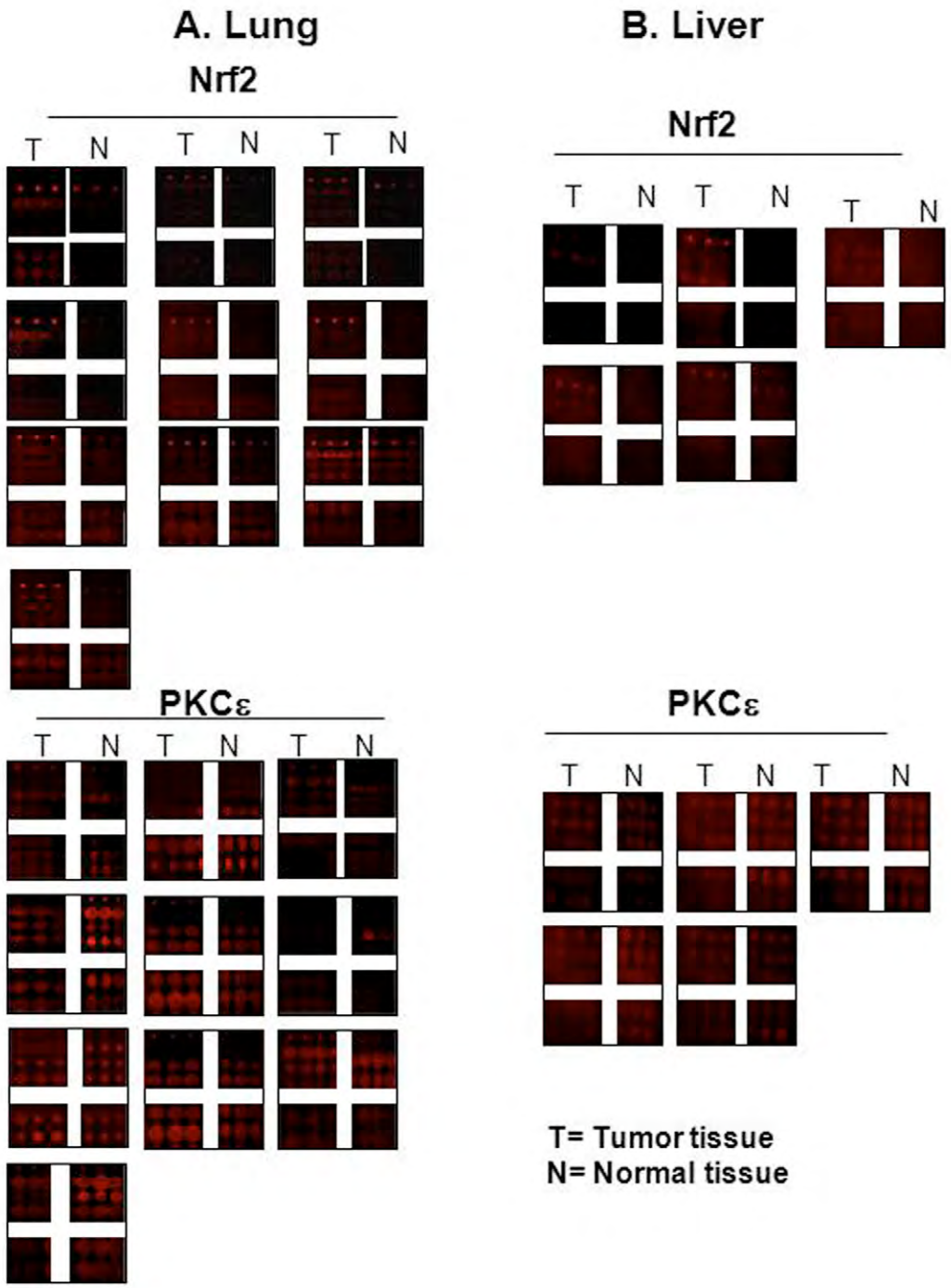

Figure S8. (A&B) Nrf2 and PKCε level analysis from different normal and tumor human tissues by protein microarray analysis. SomaPlex reverse phase protein microarray slides of human lung tumor and normal tissues and human liver tumor and normal tissues were obtained from Protein Biotechnology (Romona, CA). The slides were subjected to immunostaining for Nrf2 and PKCε antibodies, followed by anti-rabbit Alexa Fluor-594 conjugated secondary antibodies (Invitrogen). After immunostaining, slides were observed under a Nikon fluorescence microscope and photographed (upper and lower panels).

Table S1. Sequences of the PCR primers used for construction of various mutants of INrf2

Construct name	Sequence of the PCR primer used
pcDNAINrf2S602A-V5	FP : CC CGC ATG ACA TCT GGC CGC GCT GGG GTG GGT GT RP: GCG GCC AGA TGT CAT GCG GGT CAC CTC ACT CCA GG
pcDNAINrf2S602C-V5	FP: GCG GCC AGA TGT CAT GCG GGT CAC CTC ACT CCA GG RP:GCG GCC AGA TGT CAT GCG GGT CAC CTC ACT CCA GG
pcDNAINrf2S599A-V5	FP:GT GAG GTG ACC CGC ATG ACA GCT GGC CGC AGC GG RP:TGT CAT GCG GGT CAC CTC ACT CCA GGT ATC ACT GT
pcDNAINrf2S599C-V5	FP:AGT GAG GTG ACC CGC ATG ACA TGT GGC CGC AGC GG RP:TGT CAT GCG GGT CAC CTC ACT CCA GGT ATC ACT GT
pcDNAINrf2 S599AS602A-V5	FP:GTG ACC CGC ATG ACA GCT GGC CGC GCC GGG GTG GGT GTG RP:TGT CAT GCG GGT CAC CTC ACT CCA GGT ATC ACT GT
pcDNAINrf2 S599CS602C-V5	FP:GTG ACC CGC ATG ACA TGT GGC CGC TGC GGG GTG GGT GTG RP: RP:TGT CAT GCG GGT CAC CTC ACT CCA GGT ATC ACT GT
pcMxFlag-PKCε	FP:AGCGGC AGATCT ATG GTA GTG TTC AAT GGC CTT CTT RP AGGATT CTCGAG TCA GGG CAT CAG GTC TTC ACC AA
INrf2 S602A -HIS-Tagged	FP:ATG ACA TCT GGC CGC GCT GGG GTG GGT GTG GCC RP:GGC CAC ACC CAC CCC AGC GCG GCC AGATGT CAT