**Figure 1 Supplement 1**

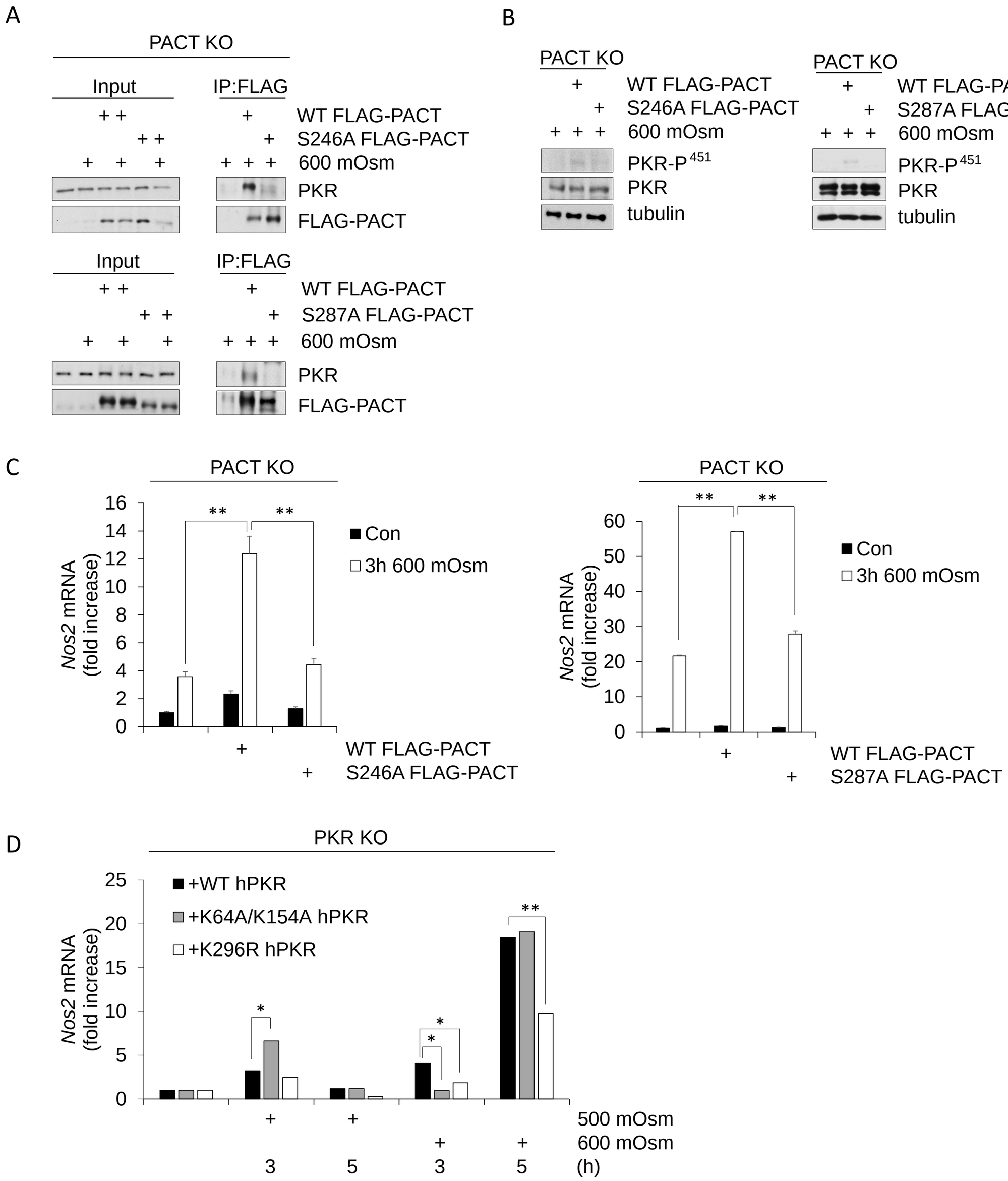


**Figure 1 Supplement 1.** PACT Ser-246 and Ser-287 are necessary for PKR interaction and downstream inflammatory signaling. (A) PACT KO MEFs were reconstituted with FLAG-WT PACT or FLAG-S246A mutant PACT constructs, then treated with 600 mOsm sucrose for 3 h. Proteins from total cell extracts or co-immunoprecipitated with the FLAG antibody were analyzed via Western blot. (B) PACT KO MEFs were reconstituted with FLAG-WT PACT, FLAG-S246A, or FLAG-S287A mutant PACT constructs, then treated with 600 mOsm sucrose for 3 h. Total lysates were analyzed via Western blot. (C) PACT KO MEFs were reconstituted with FLAG-WT PACT or FLAG-S246A mutant PACT constructs, then treated with 600 mOsm sucrose for 3 h. RNA was isolated, and *Nos2* transcript levels were analyzed via qPCR. (D) PKR KO MEFs reconstituted with wild type PKR, PKR mutated at the RNA-binding residues (K64R/K154R), or kinase activity-deficient PKR (K296R) were treated with 500 or 600 mOsm sucrose for the indicated durations. RNA was isolated, and *Nos2* transcript levels were analyzed via qPCR.