

Figure S1 Conformational changes of IRF-3 upon phosphorylation and intermolecular interactions between the phosphorylated IRF-3 dimer. (A and B) Superposition of the phosphorylated human IRF-3/CBP and auto-inhibited IRF-3 (PDB: 1QWT) showing the conformational changes of IRF-3 upon phosphorylation. The IRF-3/CBP dimer is colored the same as in Figure 1B. Auto-inhibited IRF-3 is colored orange. (C to F) Intermolecular interactions between phosphorylated human IRF-3 molecules.

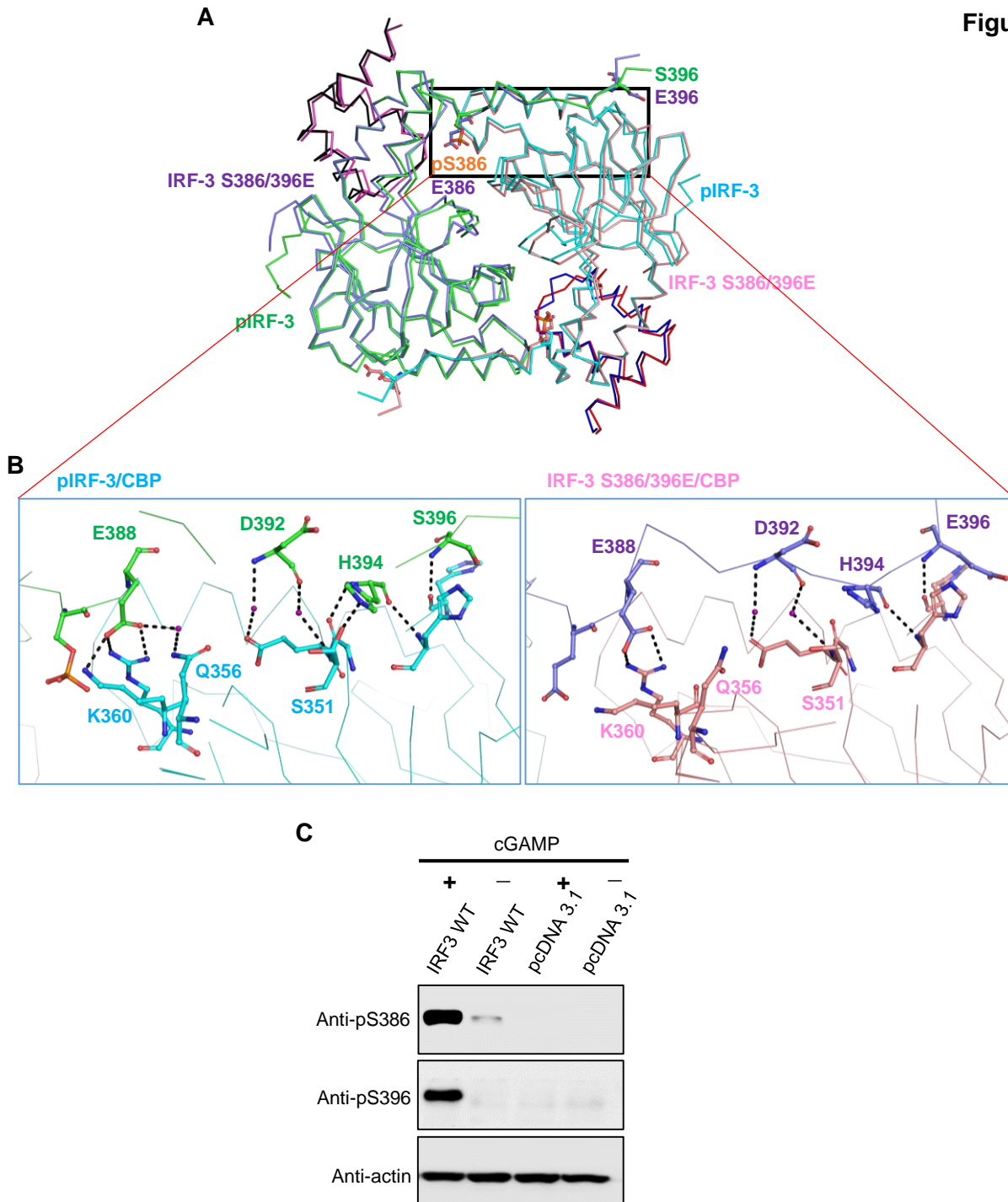


Figure S2 Comparison of structures of phosphorylated human IRF-3 and its S386/396E mutant bound to CBP and phosphorylation of Ser386 and Ser396 in cells. (A) Superposition of structures of phosphorylated human IRF-3/CBP complex and phosphomimetic S386/396E mutant bound to CBP (PDB: 5JEM). Phosphorylated IRF-3 are in green and cyan. The phosphomimetic mutant are in purple and pink. (B) The comparison of intermolecular interactions downstream of E386 and pS386 within phosphorylated and phosphomimetic IRF-3 dimers. (C) Western blot showing the phosphorylation of Ser386 and Ser396 in HEK293T cells transfected with wild-type IRF-3.

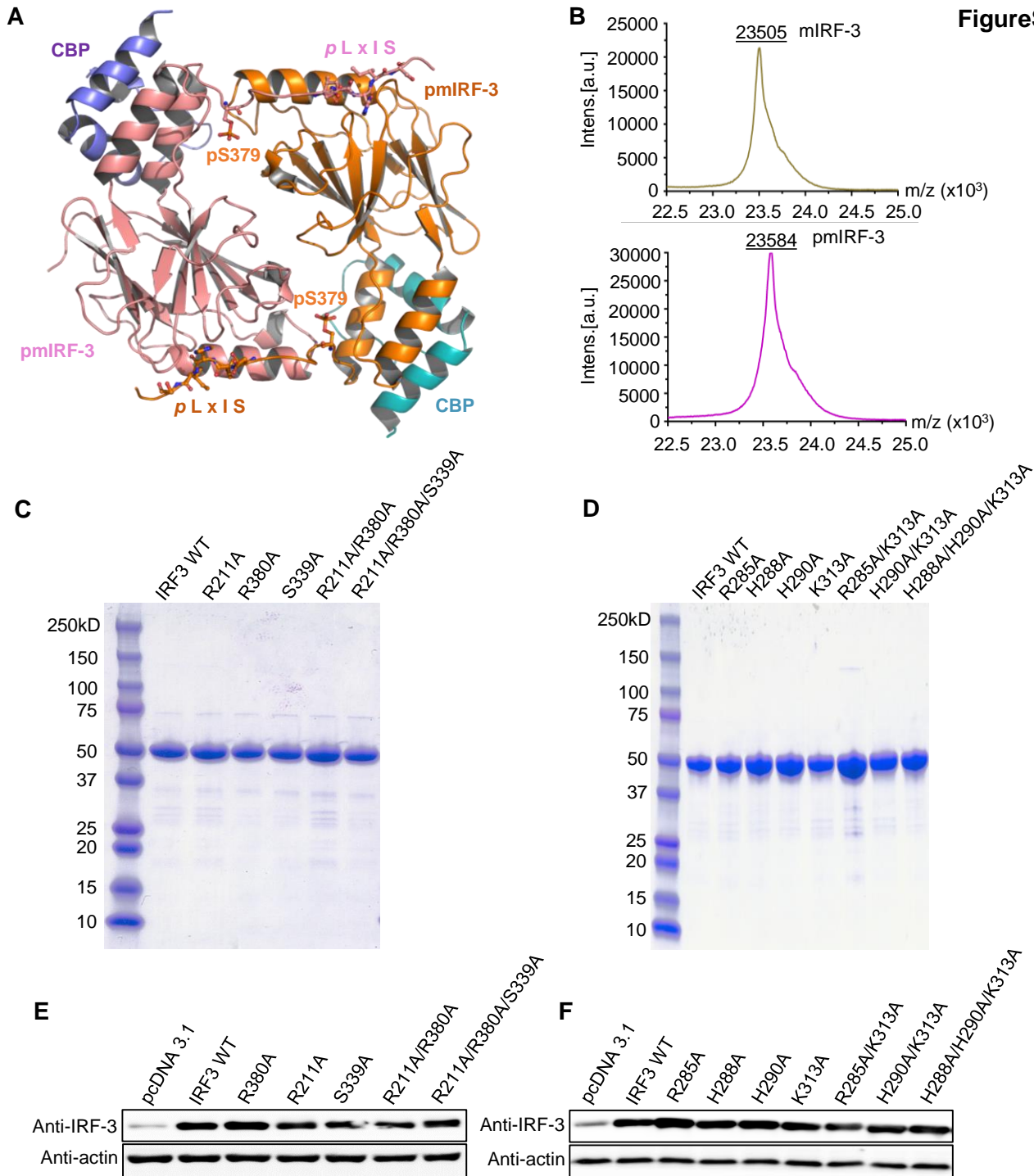


Figure S3 Structure of phosphorylated mouse IRF-3 bound to CBP and SDS-PAGE analyses of purified human IRF-3 mutants and expression of IRF-3 mutants in cells. (A) The structure of phosphorylated mouse IRF-3/CBP dimer. Phosphorylated IRF-3 are colored pink and orange. CBP are in purple and teal. **(B)** MS analyses of mouse IRF-3 (residues 184-390) before and after TBK1 phosphorylation. **(C and D)** Gradient gel electrophoreses of purified human IRF-3 mutants. **(E and F)** Western blot showing the expression of IRF-3 mutants in HEK293T cells.

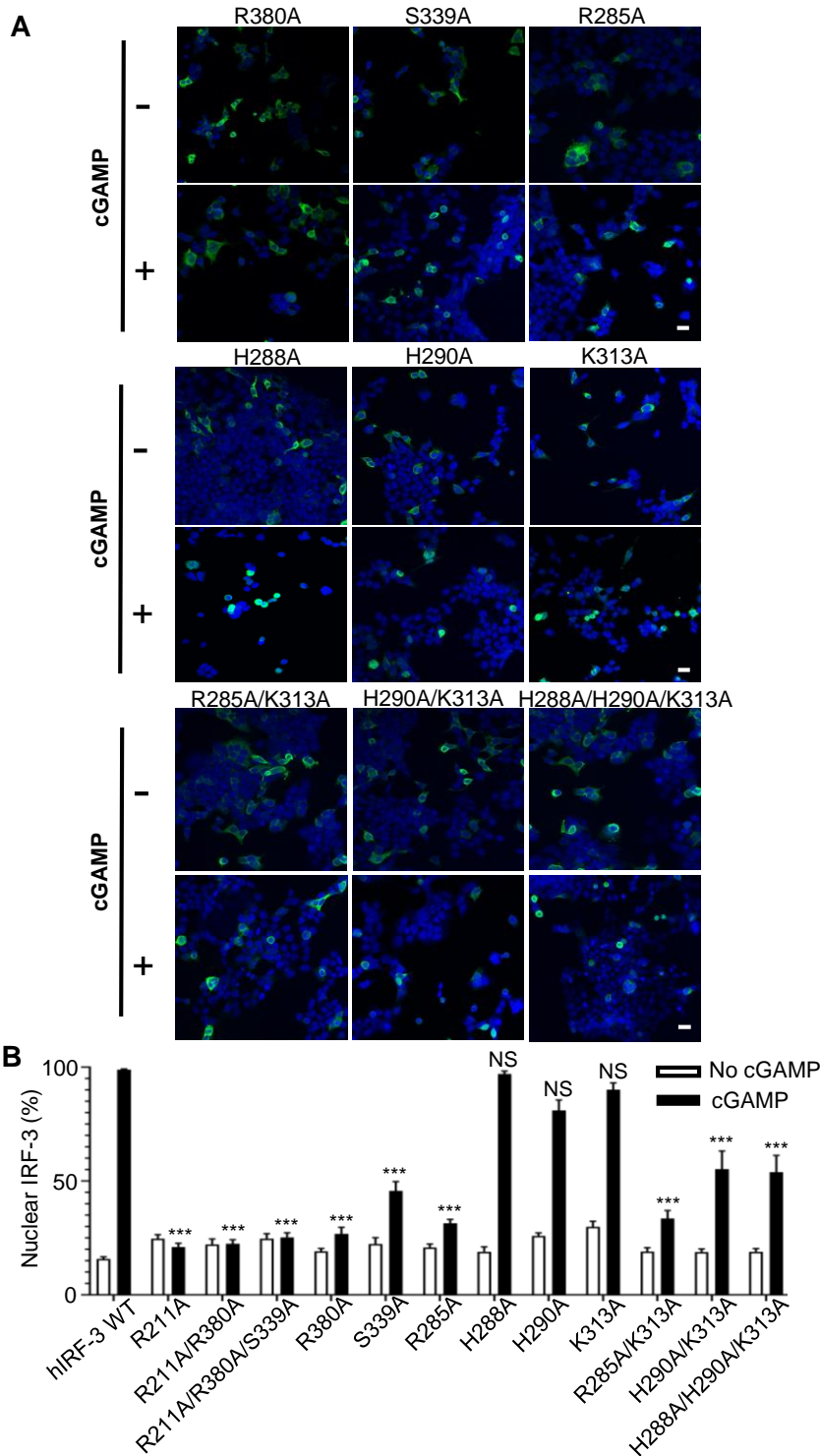


Figure S4 Mutations of residues interacting with pSer386 and pSer396 of hIRF-3 affect IRF-3 nuclear localization and quantification of nuclear translocation of hIRF-3 in HEK293T cells. (A) Confocal microscopy of HEK293T cells transfected with wild-type or IRF-3 mutants upon cGAMP stimulation. Scale bars, 20 μ m. (B) Nuclear hIRF-3 in wild-type or IRF-3 mutants with or without cGAMP stimulation is quantified by ImageJ. Statistical analysis was performed by 2-way ANOVA with Tukey's multiple comparisons test. *** $P < 0.001$, NS ($P > 0.05$).