

Supplementary Materials for
Phosphoinositide 3-Kinase γ Inhibits Cardiac GSK-3 Independently of Akt

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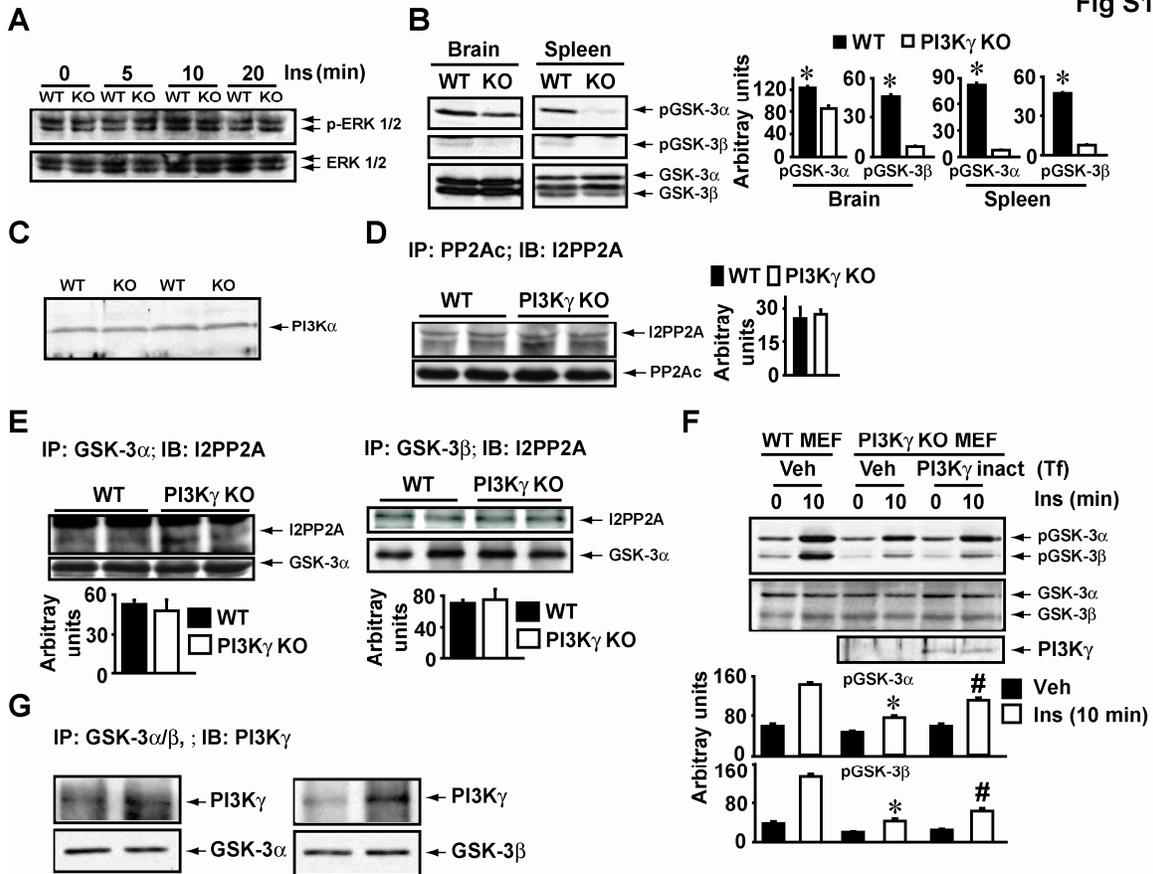


Figure S1: Role of the GSK-3-PP2Ac interaction in dephosphorylation of GSK-3

(A) Cardiac lysates from WT or PI3K γ KO mice were immunoblotted with anti-phospho-ERK1/2 antibody following insulin challenge. The blot was stripped and re-probed with anti-ERK1/2 antibody as a loading control. $n = 6$ mice per genotype. (B) Lysates of brain and spleen tissues from WT and PI3K γ KO mice were immunoblotted with anti-phospho-GSK-3 (GSK-3 α and β) antibody. The blots were stripped and re-probed for total GSK-3 α and β (lower panel). Amalgamated densitometry of phosphorylated GSK-3 α or β normalized to total GSK-3 α or β is presented in the bar graphs. $n = 5-6$ mice per genotype and each lane represents one mice. $*P < 0.001$ compared to PI3K γ KO. (C) Cardiac lysates from WT and PI3K γ KO mice were immunoblotted with anti-PI3K α

antibody. $n = 6$ mice per genotype. **(D)** PP2Ac immunoprecipitates from cardiac lysates of PI3K γ KO or WT mice were immunoblotted for co-immunoprecipitating I2PP2A. $n = 6$ mice per genotype. Amalgamated densitometry for co-immunoprecipitating I2PP2A normalized to PP2Ac is presented in the bar graph. **(E)** GSK-3 α and β immunoprecipitates from cardiac lysates of PI3K γ KO or WT mice were immunoblotted for co-immunoprecipitating I2PP2A. $n = 6$ mice per genotype. Amalgamated densitometry of co-immunoprecipitating I2PP2A normalized to GSK-3 α or β is presented in the bar graphs. **(F)** Cell lysates from PI3K γ KO MEFs transfected with Veh or PI3K γ_{inact} were immunoblotted with anti-phospho-GSK-3 (GSK-3 α and β). The blots were stripped and reprobed for GSK-3 α and β . Amalgamated densitometry of phosphorylated GSK-3 α or β normalized to total GSK-3 α and β is presented in the bar graphs. $n = 5-6$ independent experiment in duplicates. $*P < 0.001$ compared to WT MEFs. $\#P < 0.05$ compared to Veh-PI3K γ KO MEFs. **(G)** GSK-3 α and β immunoprecipitates from cardiac lysates of PI3K γ_{inact} /PI3K γ KO mice were immunoblotted for co-immunoprecipitating PI3K γ . $n = 6$ mice per genotype.

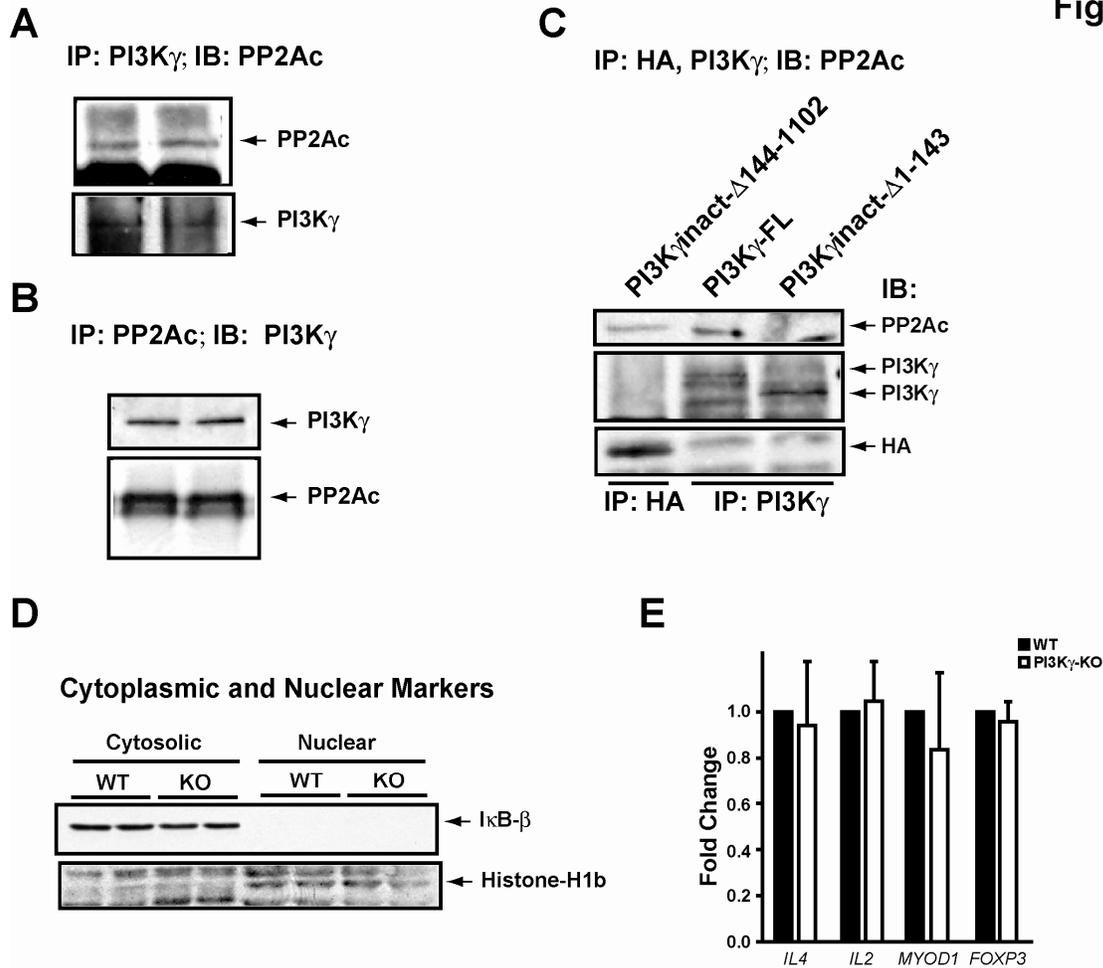


Figure S2: Interaction of PI3K γ with PP2Ac and GSK-3

(A) PI3K γ immunoprecipitates from lysates of HEK293 cells were immunoblotted for co-immunoprecipitating PP2Ac. $n = 5$ independent experiments in duplicates. (B) PP2Ac immunoprecipitates from cardiac lysates of PI3K γ_{inact} /PI3K γ KO mice were immunoblotted for co-immunoprecipitating PI3K γ . $n = 5$ mice per genotype. (C) HA or PI3K γ immunoprecipitates from PI3K γ KO MEFs transfected with deletion constructs of PI3K γ_{inact} were blotted for co-immunoprecipitating PP2Ac. The blots were stripped and re-probed for PI3K γ or HA. $n = 5$ independent experiments in duplicates. (D) The cytoplasmic and nuclear fractions of cardiac lysates from WT and PI3K γ KO mice were

immunoblotted with a cytoplasmic marker ($\text{I}\kappa\text{B-}\beta$) or a nuclear marker (histone H1b). $n = 6$ mice per genotype. (E) Expression of NFATc3 regulated genes *IL4*, *IL2*, *MYOD1* and *FOXP3* in WT and PI3K γ KO mice was measured using real time RT PCR and represented as fold change over WT. $n = 5$ mice per genotype.