Molecular Pharmacology

The large isoforms of AKAP18 mediate the Phosphorylation of Inhibitor-1 by PKA and the inhibition of PP1 Activity

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Supplementary Figure Legends:

Supplementary Figure 1:

Isolated rat heart extract was incubated with either GST-I-1 or control GST-protein. mAKAP was detected by western blot (left) and total protein by Ponceau stain (right) n=3.

Supplementary Figure 2:

A. GST-I-1 fusion protein (3 μg) was incubated with HEK293 cell lysate extracted from prepared from cells expressing AKAP18γ, or AKAP18γ 1-268. Protein stain corresponds to western blots shown in Figure 4B. B. HEK293 cells expressing I-1 and full-length AKAP18γ were stimulated with 1 μM Isoproterenol for 5 minutes after a one hour preincubation with H-89 (10 μM). Whole cell lysate were analyzed by western blot using antibodies against phospho-I-1 Thr-35 (upper panel), I-1 (middle panel) and GFP for AKAP18 (lower panel).

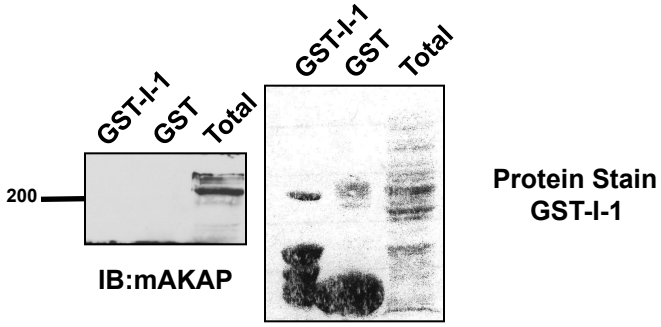
Supplementary Figure 3:

Protein complexes were immunoprecipitated from lysates prepared from HEK293 cells expressing GFP-tagged AKAP18γ using either a PP1 or PP2A catalytic subunit antibody. Phosphatase activity in the immunoprecipitates was performed as described

in the Methods section. Immunoprecipitations correspond to western blots shown in Figure 5E.

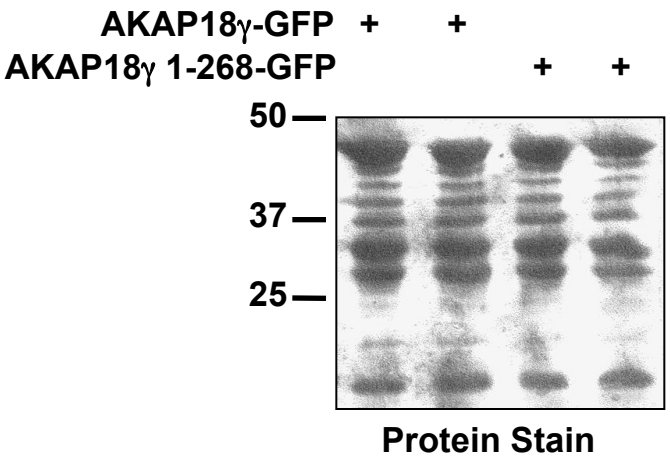
Supplementary Figure 4:

Protein complexes were immunoprecipitated from lysates prepared from HEK293 cells transfected with I-1 plus and minus GFP-AKAP18γ using antibodies to AKAP18, PKA-RII and PP1. A). AKAP18 was immunoprecipitated using an antibody specific for the anchoring protein followed by western blot analysis using an antibody that recognized the GFP tag on AKAP18. Proteins in the extracts are shown in the bottom panels. B). The regulatory subunit of PKA was immunoprecipitated and PKA activity was determined on the immunoprecipitate, demonstrating equal amounts of PKA was immunoprecipitated under each condition. Total protein in the extracts is shown in the bottom panels. C) Phosphatase assay performed on PP1 immunoprecipitates in the presence and absence of AKAP18. Similar amounts of phosphatase were immunoprecipitated under each condition. Total protein in the extracts is shown in the bottom panel. Total protein is 5% of input. n=3 for each.



Supplemental Figure 1

A.



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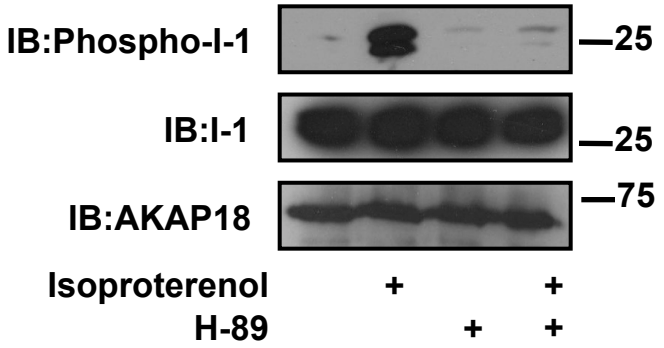
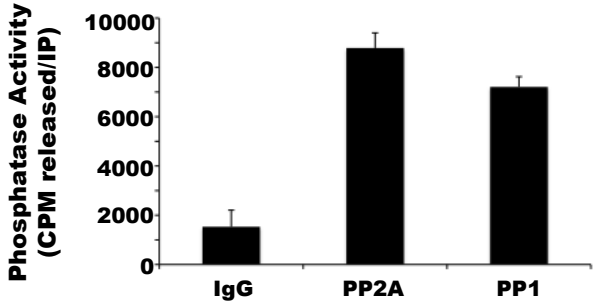
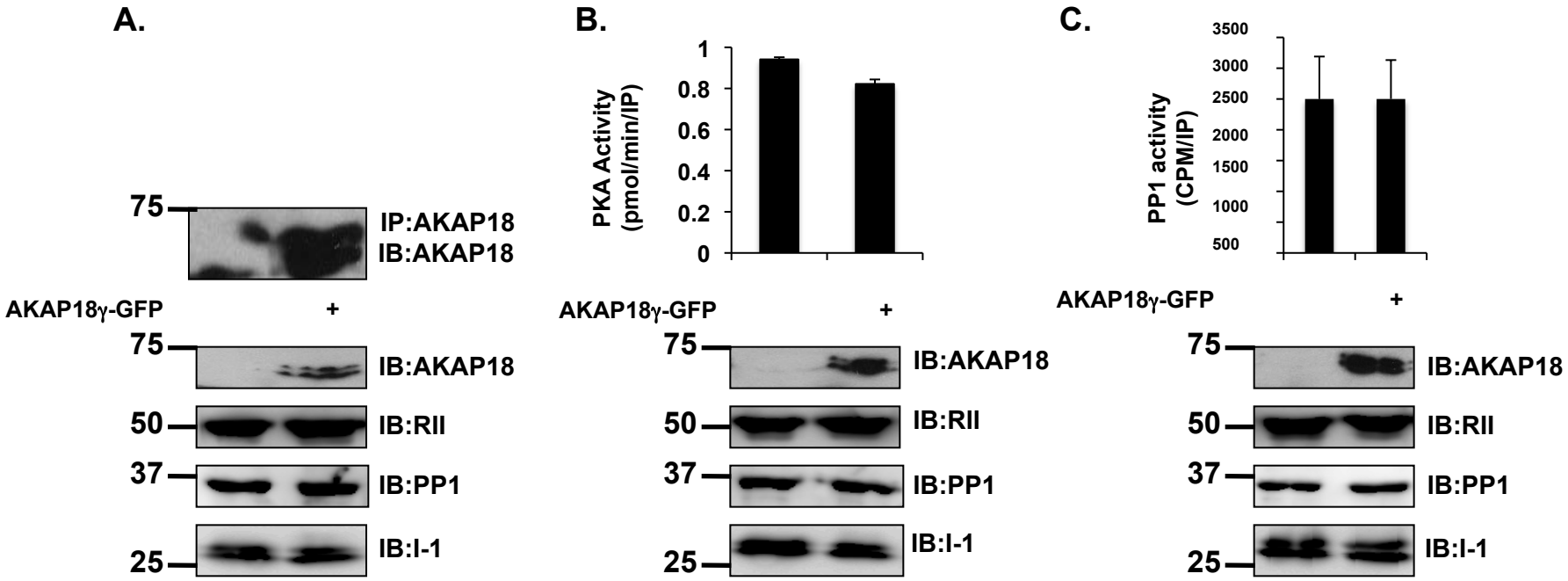


Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4