

Supplementary Materials for **mAKAP Compartmentalizes Oxygen-Dependent Control of HIF-1 α**

Wei Wong, April S. Goehring, Michael S. Kapiloff, Lorene K. Langeberg, John D. Scott*

*To whom correspondence should be addressed. E-mail: scottjdw@u.washington.edu

Published 23 December 2008, *Sci. Signal.* **1**, ra18 (2008)
DOI: 10.1126/scisignal.2000026

This PDF file includes:

Fig. S1. PHD1 does not interact with mAKAP.

Fig. S2. pVHL and Siah2 bind to mAKAP.

Fig. S3. Mapping of mAKAP-binding regions.

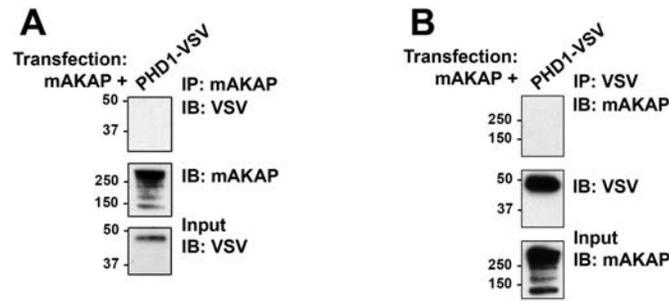


Fig. S1. PHD1 does not interact with mAKAP. The binding of epitope-tagged PHD1 to mAKAP was evaluated by two methods. **(A)** mAKAP immunoprecipitates were examined for the coimmunoprecipitation of VSV-tagged PHD1 in HEK 293 cells. PHD1 was not detected in the mAKAP complex (top panel). The amount of mAKAP is indicated (middle panel), and PHD1 was detected in Western blots of cell lysates (bottom panel). **(B)** PHD1-VSV immune complexes were screened for the coimmunoprecipitation of mAKAP. The anchoring protein was not detected by Western blotting (top panel). The amount of PHD1 is indicated (middle panel), and mAKAP was detected in the Western blot of the cell lysate (bottom panel). Numbers to the left of each panel indicate molecular weight markers (in kD).

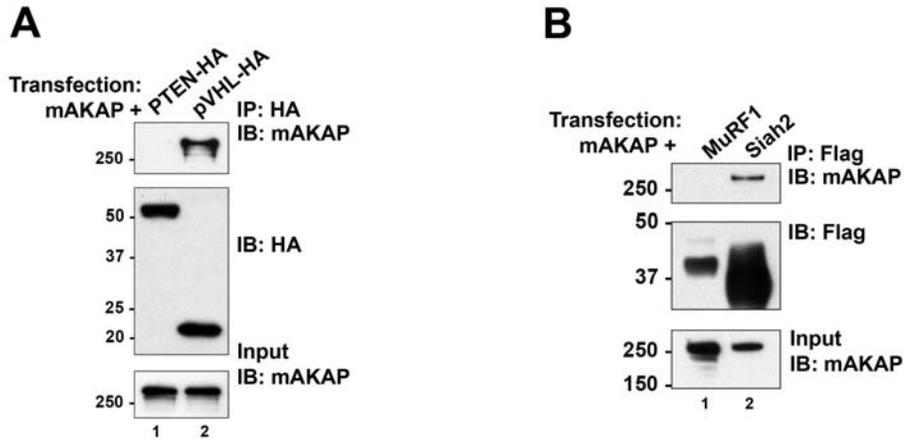


Fig. S2. pVHL and Siah2 bind to mAKAP. HEK 293 cells were cotransfected with plasmids encoding mAKAP and either (A) HA-tagged PTEN or HA-tagged pVHL or (B) FLAG-tagged MuRF1 or FLAG-tagged Siah2. (A) Samples immunoprecipitated with an antibody against the HA tag (indicated above each lane) were screened for the presence of mAKAP by Western blot analysis (top panel). The amount of each HA-tagged protein is indicated (middle panel). mAKAP was detected in cell lysates by Western blotting (bottom panel). (B) FLAG-tagged MuRF1 and FLAG-tagged Siah2 (indicated above each lane) were examined for their binding to mAKAP by Western blot analysis (top panel). The amount of each FLAG-tagged protein is indicated (middle panel). mAKAP was detected in Western blots of cell lysates (bottom panel). Numbers to the left of each panel indicate molecular weight markers (in kD).

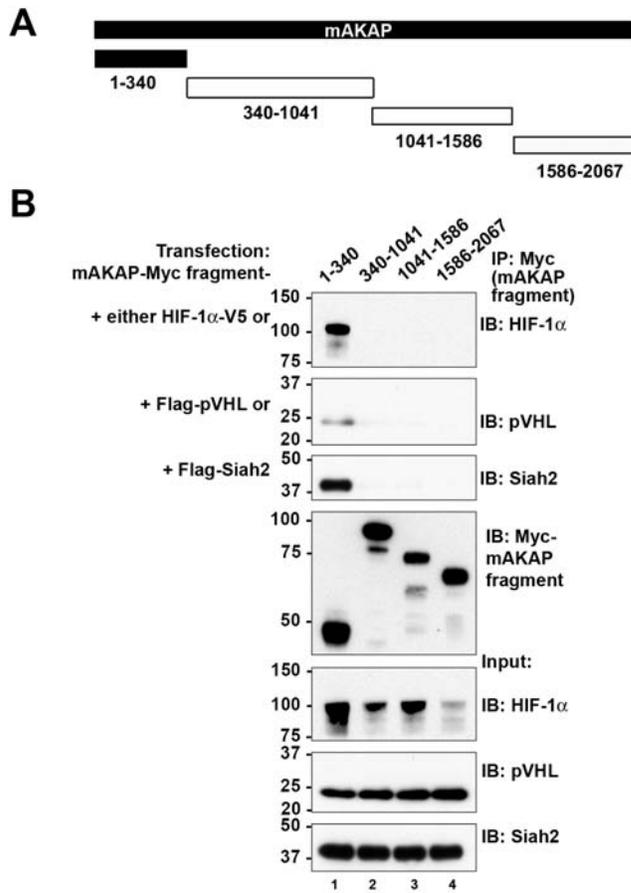


Fig. S3. Mapping of mAKAP-binding regions. (A) Diagram depicting the mAKAP fragments used in mapping studies. (B) HIF-1 α , pVHL, and Siah2 bound to the N-terminus of mAKAP in HEK 293 cells. Four mAKAP fragments (indicated above each lane) were examined for their interactions with V5-tagged HIF-1 α , FLAG-tagged pVHL, and FLAG-tagged Siah2. These proteins coimmunoprecipitated with the 1-340 fragment of mAKAP (top, second, and third panels). The mAKAP fragments were immunoprecipitated with an anti-Myc antibody (center panel). The bottom three panels show the abundance of each expressed protein in the cell lysates. Numbers to the left of each panel indicate molecular weight markers (in kD).