Supplemental Information

Asp1 from *Schizosaccharomyces pombe* Binds a [2Fe-2S]$^{2+}$ Cluster Which Inhibits Inositol Pyrophosphate 1-Phosphatase Activity.

Huanchen Wang,[†] Vasudha S. Nair,[†] Ashley A. Holland,[‡] Samanta Capolicchio,[§] Henning J. Jessen,[§] Michael K. Johnson,[‡] and Stephen B. Shears*,[†]

[†]Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, 101 T.W. Alexander Drive, Research Triangle Park, North Carolina, 27709, USA. [‡]Department of Chemistry and Center for Metalloenzyme Studies, University of Georgia, Athens, GA 30602. [§]Department of Chemistry, University of Zurich (UZH), Winterthurerstrasse 190, 8057 Zurich, Switzerland.

*Corresponding author: email shears@niehs.nih.gov

Supplementary Table 1. A list of Mutagenic Primers

C448S 5'-CTACCAACCTTGCTACAGAACTCAAA**TCC**GAAGATATCAACAAGTTAAAACAGTTACGC
      3'-GCGTAACTGTTTTAACTTGTTGATATCTTC**GGA**TTTGAGTTCTGTAGCAAGGTTGGTAG

C523S 5'-GTTTCATAAGGACCTGTATATTATGAATCGTGAT**TCC**CTGAAAGATGTCGAAATATACACATCTTCTG
      3'-CAGAAGATGTGTATATTTCGACATCTTTCAG**GGA**ATCACGATTCATAATATACAGGTCCTTATGAAAC

C607S 5'-CCAGAAAACATGCCCAAGCCT**TCT**GAAGTAATGCAACAAGTTGTTCAACTCATG
      3'-CATGAGTTGAACAACTTGTTGCATTACTTC**AGA**AGGCTTGGGCATGTTTTCTGG

C643S/C644S 5'-GTTGAGCAAGTTCAATCGCGTTGG**TCTTCT**AATGAAAATCCTGCGCTCTTTAGGG
      3'-CCCTAAAGAGCGCAGGATTTTCATT**AGAAGA**CCAACGCGATTGAACTTGCTCAAC

C663S 5'-GTGGGAGAAGTTGTTTAGCGAATTT**TCT**GACTCTGAGAAAGCAGACCC
      3'-GGGTCTGCTTTCTCAGAGTC**AGA**AAATTCGCTAAACAACTTCTCCCAC

C814S 5'-CTATACCTTGCTTAAT**TCT**ATACTTGAATCAGGACTACCTATG
      3'-GGTAGTCCTGATTCAAGTAT**AGA**ATTAAGCAAGGTATAGATATG

C839S 5'-TCTCACTCAAATA**TCC**TTTGAGTTATTCGAGAGGACTAATC
      3'-GTCCTCTCGAATAACTCAAA**GGA**TATTTGAGTGAGATAATC

C864S 5'-CTCTCACCAGGT**TCT**TATGCCCAATGTCCATTGG
      3'-GGACATTGGGCATA**AGA**ACCTGGTGAGAGTG

C868S 5'-CCAGGTTGTTATGCCCAA**TCT**CCATTGGATATGAATCTTG
      3'-TATCCAATGG**AGA**TTGGGCATAACAACCTGGTGAG

C879S 5'-GTCCATTGGATATGAATCTTGATGCTAAACAC**TCT**ATTAGCGTTTCACCTCGCAGGTC
      3'-GACCTGCGAGGTGAAACGCTAAT**AGA**GTGTTTAGCATCAAGATTCATATCCAATGGAC

C905S 5'-GTTTATAACGAAAACCGAGGACCTC**TCC**AACTCAGTTCATCTTCCCAAAAGG
      3'-CCTTTTGGGAAGATGAACTGAGTT**GGA**GAGGTCCTCGGTTTTCGTTATAAAC

H807A 5'-GTACTTTACTAAAGAGTCT**GCT**CTATCTATACCTTGCTTAATTGTATAC
      3'-CAATTAAGCAAGGTATAGAT**AGC**AGACTCTTTAGTAAAGTACATTCTTG

Pairs of complementary primers were designed as shown below, with mutagenic codons underlined and in bold font. The sequences of each mutant were verified.

Supplementary Figure S1. Multiple Sequence Alignment of hPPIP5K2, hPPIP5K1 and Asp1

A

```
hPPIP5K2    363   EAEDIPIVPTTSGTMMELRCVIAVIRHGDRTPKQKMKMEVRHQKFFDLFEKCDGYKSGKLKLKKPKQLQE
hPPIP5K1    374   EAEDIPIVPTTSGTMMELRCVIAIIRHGDRTPKQKMKMEVKHPRFFALFEKHGGYKTGKLKLKRPEQLQE
Asp1        371   SVQEVLNPPPRESEAWRLKSLVGVLRHADRTPKQKFKFSFTSDPFVKLLQGHT-----EEVILRNEQLNS

Consensus         php-l..sPspptphhcL+tlltllRHtDRTPKQKhKhphpp..Fh.Lhp.hs.....cb.lb+scQLpp


hPPIP5K2    433   VLDIARQLLMELGQNNDSEIEENKPKLEQLKTVLEMYGHFSGINRKVQLTYLPHGCPKTS-SEEEDSRRE
hPPIP5K1    444   VLDITRLLLAELEKEPGGEIEEKTGKLEQLKSVLEMYGHFSGINRKVQLTYYPHGVKASNEGQDPQRETL
Asp1        436   VLAATNLATEL-------KCEDINKLKQLRLALETKKDLPGTKVQLKPAYSPEGKL-------------

Consensus         VLshhpbhh.b........b.Ep.sKLcQL+.hLEhb.chsGhp.plp.hY.PcG...............


hPPIP5K2    502   EPSLLLVLKWGGELTPAGRVQAEELGRAFRCMYPGGQGDYAGFPGCGLLRLHSTYRHDLKIYASDEGRVQ
hPPIP5K1    514   APSLLLVLKWGGELTPAGRVQAEELGRAFRCMYPGGQGDYAGFPGCGLLRLHSTFRHDLKIYASDEGRVQ
Asp1        485   -LKLQLIIKWGGEFTHSARYQSKDLGEQFHK--------------DLYIMNRDCLKDVEIYTSSERRVS

Consensus         ..pLbLllKWGGEhT.ttRhQtc-LGc.F+................sLh.hppsh.+DlcIYhSsE.RVp


hPPIP5K2    572   MTAAAFAKGLLALEGELTPILVQMVKSANMNGLLDSDSDSLSSCQQRVKARLHEILQKDRDFTA-EDYEK
hPPIP5K1    584   MTAAAFAKGLLALEGELTPILVQMVKSANMNGLLDSDGDSLSSCQHRVKARLHHILQQDAPFGP-EDYDQ
Asp1        539   ASAEIFAMAFLEQET-IPSDLLKVRKD-----LLD-DSNAAKDTMDKVKKHLKSLLRVGDTARKEFTWPE

Consensus         hoA.hFAbthL.bEs.lss.Llph.Ks.....LLD.Dtsthpshbp+VK.+L+plLp.s.sh...bs@.p


hPPIP5K2    641   LTPSGSISLIKSMHLIKNPVKTCDKVYSLIQSLTSQIRHRMEDPKSSDIQLYHSETLELMLRRWSKLEKD
hPPIP5K1    653   LAPTRSTSLLNSMTIIQNPVKVCDQVFALIENLTHQIRERMQDPRSVDLQLYHSETLELMLQRWSKLERD
Asp1        602   NMPKPCEVMQQVVQLMKYHRAVMRENFIILGPEVEQVQSR----------WCCNENPALFRERWEKLFSE

Consensus         .hPp.t.shbpshplhp....hhcps@.ll.sbhpQlppR..........hhhsEs..Lh.pRWpKLbp-
```

```
hPPIP5K2    711    FKTKNGRYDISKIPDIYDCIKYDVQHNGSLK----------------------------------------
hPPIP5K1    723    FRQKSGRYDISKIPDIYDCVKYDVQHNGSLG----------------------------------------
Asp1        662    FCDS-EKADPSKVSELYDTLKYDALHNRQFLERIFTPYQYLKLPQSPSLIAKEPPQRTDSNGNLVGMTGA

Consensus          F.pp..+hD.SKls-lYDhlKYDhbHN.ph.........................................


hPPIP5K2    742    --------LENTMELYRLSKALADIVIPQEYGITKAEKLEIAKGYCTPLVRKIRSDLQRTQDDDTVNKLH
hPPIP5K1    754    --------LQGTAELLRLSKALADVVIPQEYGISREEKLEIAVGFCLPLLRKILLDLQRTHEDESVNKLH
Asp1        731    NTNHTERPLEKLYELYDLAKVLFDFVSPQEYGIEPKEKLEIGLLTSVPLLRQIIHDIKEA----------

Consensus          ........Lp.hhELhcLtKhLhDhV.PQEYGIp..EKLEIt..hthPLlRpI..Dlpch..........


hPPIP5K2    804    PVYSRGVLSPERHVRTRLYFTSESHVHSLLSILRYGALCNESKDEQWKRAMDYLNVVNELNYMTQIVIML
hPPIP5K1    816    PLYSRGVLSPGRHVRTRLYFTSESHVHSLLSVFRYGGLLDETQDAQWQRALDYLSAISELNYMTQIVIML
Asp1        791    --------RDSDHASTRMYFTKESHIYTLLNCILESGLPMKLPRN----------QIPELDYLTQICFEL

Consensus          ........ps.cHhpTRhYFTpESHl@oLLshh.bttL..c..c............lsELsYhTQIhhbL


hPPIP5K2    874    YEDPNKDLSSEERFHVELHFSPGAKGCEEDKNLPSGYGYRPASRENEGRRPFKIDNDDEPHTSKRDEVDR
hPPIP5K1    886    YEDNTQDPLSEERFHVELHFSPGVKGVEEEGSAPAGCGFRPASSENEEMKTNQGSMENLCPGKASDEPDR
Asp1        843    FERTN--PSGNKEFSVRITLS-----------PGCYAQCPLDM--------------------------

Consensus          @Ecss....tpccFpVclhhS............Ptthtb.Phs...........................


hPPIP5K2    944    AVILFKPMVSEPIHIHRKSPLPRSRKTATNDVVSENA--------------------------------
hPPIP5K1    956    AL-QTSPQPPEGPGLPRRSPLIRNRKAGSMEVMNMQCTGNLDLIPLRGRRCRRSGDLPQPSLAIGLQPRA
Asp1        873    -----------------------NLDA--------------------------------------------

Consensus          ...............................phss..................................


hPPIP5K2    981    ------------------------------------------------------NYLRT------------
hPPIP5K1    1025   VSTTHLASCTQVLSETSSSRPGGYRLFSSSRPPTEMKQSGLGSQCTGLFSTTVLGGSSSAPNLQDYARSH
Asp1               ---------------------------------------------------------------------

Consensus          .....................................................................
```

```
hPPIP5K2    986   --------------------PRTLVEQKQNPTVGFELYSMVPSICPLETLHNALSLKQVDEFLASIASP
hPPIP5K1    1095  GKKLPPASLKHRDELLFVPAVKRFSVSFAKHPTNGFEGCSMVPTIYPLETLHNALSLRQVSEFLSRVCQR
Asp1        877   ---------------------------KHCISVSPRR---SLTRHLDLQQFITKTEDLCNS
                                                        *                        *
Consensus         ...............................c.h..Vss.....oLhphLsLpQh.pbh.pltp..


hPPIP5K2    1035  SSDVPRKTAEISSTALRSSPIMRKKVSLNTYTPAKIL
hPPIP5K1    1165  HTDAQAQ-----ASAALFDSMHSSQASDNPFSPPRTL
Asp1        908   V----------------------LPKRFIPVNI-

Consensus         ..........................p.@.Psph.
```

B

```
hPPIP5K2    917   GAKGCEEDKNLPSGYGYRPASRENEGRRPFKIDNDDEPHTSKRDEVDR
hPPIP5K1    908   GVKGVEEEGSAPAGCGFRPASSENEEMKTNQGSMENLCPGKASDEPDR
Asp1        863   -------------GCYAQ------------------CPLDMNLDAKH
                               *                     *
Consensus         ............Gh.hb....................s....p.-sc+
```

Panel A The alignment shown is the raw output – no manual adjustments – as generated by PROMALS3D, a sequence and structure alignment server (http://prodata.swmed.edu/promals3d/promals3d.php). Residues 371 to 920 of Asp1 from *S. pombe* (Accession number O74429.1) were aligned with the corresponding regions of the human isoforms of PPIP5K1 (XP_011520555) and PPIP5K2 (XP_005271995) in a two-stage process; first we aligned the predicted, highly-structured regions up to Ser994, Ser896 and Ser861 in hPPIP5K2, hPPIP5K1 and Asp1, respectively. A second alignment was then separately performed for the remaining, largely unstructured regions. Default settings were used with the exception of (i) increasing, to a value of 1.0, the identity threshold above which fast alignment is applied, and (ii) employing PROMALS rather than MAFFT at the first alignment stage.

Panel B: The indicated section of the alignment was adjusted manually. Note the higher degree of conservation of this short Asp1 sequence with that in hPPIP5K1 compared to hPPIP5K2.

Red font depicts predicted alpha-helix, blue font denotes predicted beta-strand. The consensus symbols (where they apply to all three sequences) are those generated by the algorithm's output: fully conserved amino acids are in bold and uppercase letters; aliphatic (I, V, L) = *l*; aromatic (Y, H, W, F) = @; hydrophobic (W, F, Y, M, L, I, V, A, C, T, H) = *h*; alcohol (S, T) = o; polar residues (D, E, H,

K, N, Q, R, S, T) = p; tiny (A, G, C, S) = t; small (A, G, C, S, V, N, D, T, P) = s; bulky residues (E, F, I, K, L, M, Q, R, W, Y) = b; positively charged (K, R, H) = +; negatively charged (D, E) = -; charged (D, E, K, R, H) = c. The asterisks highlight Cys in Asp1 that are aligned with Cys in hPPIP5K1. The catalytically-essential His807 in Asp1 is underlined, to highlight its conservation in hPPIP5K1 and hPPIP5K2.