Supporting Information for:

Targeting a Cryptic Allosteric Site for Selective Inhibition of the Oncogenic Protein Tyrosine Phosphatase Shp2

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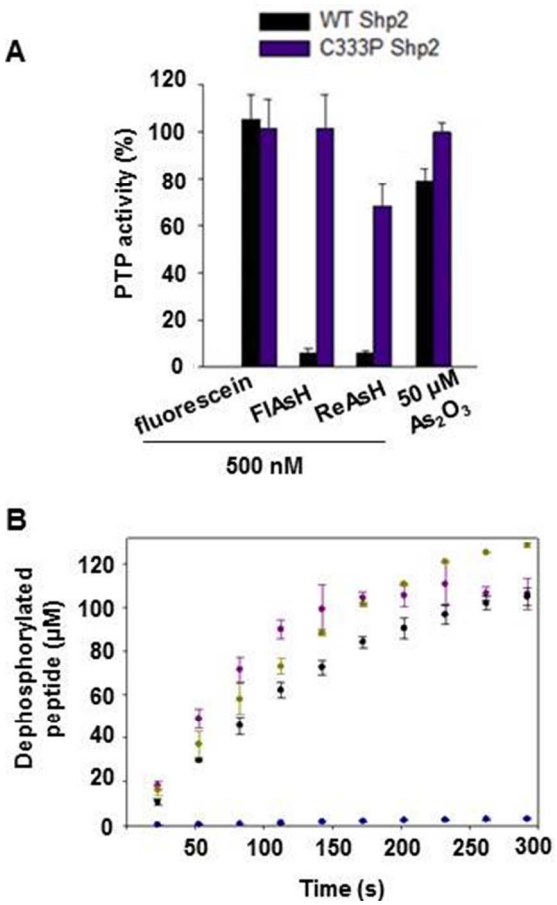

Figure S1. Desensitization of Shp2 by the C333P mutation is independent of the biarsenical compound and substrate used in the inhibition assays. (A) After incubation (120 minutes) with the indicated compounds, PTP activities of wild-type and C333P were measured with *p*NPP and normalized to no-inhibitor controls. (B) Shp2 phosphatase activity (wild-type or C333P) was measured by continuous absorbance at 282 nm[1] with a phosphopeptide substrate (DADE*p*YLIPQQG) at pH 7.0 after incubation (150 minutes) with FlAsH (225 nM) or DMSO only. The data are plotted as follows: black circles: wild-type Shp2 + DMSO; dark blue circles: wild-type Shp2 + FlAsH; dark pink circles: C333P Shp2 + DMSO: dark yellow circles: C333P Shp2 + FlAsH.

Cloning and Mutagenesis of Shp2 and Shp1 (see below for all primer sequences). A plasmid for expression of histidine-tagged full-length human Shp2 (Shp2-FL) from the pNIC28-Bsa4 vector was a generous gift from Ben Neel and Nickolay Chirgadze (Princess Margaret Cancer Center, University Health Network).[2] Plasmids pJGO001, which encodes the mouse Shp2 catalytic domain (224–539) with a C-terminal six-histidine tag, and pACB149, which encodes the human Shp1 catalytic domain (245–543) with a C-terminal six-histidine tag, were cloned using standard protocols. Briefly, PTP-encoding DNAs were amplified from template (Shp2: Open Biosystems: catalog #MMM1013-9200724; Shp1: Open Biosystems: catalog # IHS1380-8843245). The PCR products and empty pET21b vector DNA were digested with EcoRI and HindIII and gel purified. Ligation reactions containing 400 U T4 DNA ligase (New England Biolabs), 2 μL 10×ligase buffer (New England Biolabs), ~10 ng of insert, and ~10 ng of vector in a 20 μL reaction were placed in an ice bucket overnight. Ligation products were transformed into competent DH5α *E. coli* and plated on LB/Agar containing 100 μg/mL ampicillin. Ampicillin-resistant colonies were isolated, and the presence of the desired inserts was confirmed by restriction analysis and by DNA sequencing (Cornell Biotechnology Resource Center). All site-directed mutations were introduced using the Quikchange mutagenesis kit (Stratagene) according to the manufacturer's instructions. Desired mutations were confirmed by sequencing.

Primer Sequences

Cloning Primers for mouse Shp2 catalytic domain-encoding insert (restriction sites in bold)

Forward: ATCCT**GAATTCC**GCTGAAATTGAAAGCCGGGTTCGA

Reverse: ATCCT**AAGCTT**TCCTTTTCTTTTGCTTTTCTGCTCCTC

Cloning Primers for human Shp1 catalytic domain-encoding insert (restriction sites in bold)

Forward: ATCCT**GAATTC**GGGCTTCTGGGAGGAGTTTGAGAG

Reverse: ATCCT**AAGCTT**ATAGGTGATGTTCCCGTACTCCGAC

Mutagenesis Primers for C333P Shp2

Forward: GCCACTCAAGGCCCCCTGCAGAACACGGTG

Reverse: CACCGTGTTCTGCAGGGGGCCTTGAGTGGC

Expression and Purification of Shp2 and Shp1. BL21(DE3) cells containing the appropriate PTP-encoding plasmid were grown overnight at 37 ºC in LB. Cultures were diluted, grown to mid-log phase (OD_{600} = 0.5), induced with IPTG (1 mM), and shaken at room temperature overnight. The cells were harvested by centrifugation, resuspended in binding buffer (50 mM Tris at pH 7.8, 500 mM NaCl, 5 mM imidazole), and lysed by French Press at ~2000 psi. Protease Inhibitor Cocktail P8849 (Sigma Aldrich) was added to lysates of cells expressing full-length Shp2. Lysates were clarified by centrifugation, and enzyme purifications were carried out using SwellGel Nickel Chelated Discs (Pierce) according to the manufacturer's instructions. The protein solutions obtained were exchanged into storage buffer (for full-length Shp2: 100 mM Tris at pH 8.5, 100 mM NaCl, 1 mM TCEP; for the Shp2 and Shp1 catalytic domains: 50 mM 3,3-dimethyl glutarate at pH 7.0, 1 mM EDTA, 1 mM TCEP), concentrated, flash-frozen in liquid nitrogen, and stored at -80 ºC. Bradford assays and SDS-PAGE were used to estimate enzyme concentrations.

Expression and Purification of non-Shp PTPs. The His_6-tagged catalytic domains of TCPTP,[3] PTPH1,[4] FAP-1,[5] HePTP,[5] and PTP1B[6] were expressed and purified as described previously.

References

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