Supporting Information

Identification of Cryptotanshinone as an Inhibitor of Oncogenic Protein Tyrosine Phosphatase SHP2 (*PTPN11*)

Wei Liu[1,2], Bing Yu[1,2], Gang, Xu[1], Wei-Ren Xu[1,2], Mignon L. Loh[3], Li-Da Tang[2], and Cheng-Kui Qu[1]

[1] Department of Medicine, Division of Hematology and Oncology, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106, USA. [2] Tianjin Key Laboratory of Molecular Design & Drug Discovery, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China. [3] Department of Pediatrics, Division of Pediatric Hematology-Oncology, University of California, San Francisco, San Francisco, CA 94122, USA

- W. Liu, B. Yu, and G. Xu contributed equally to this paper.

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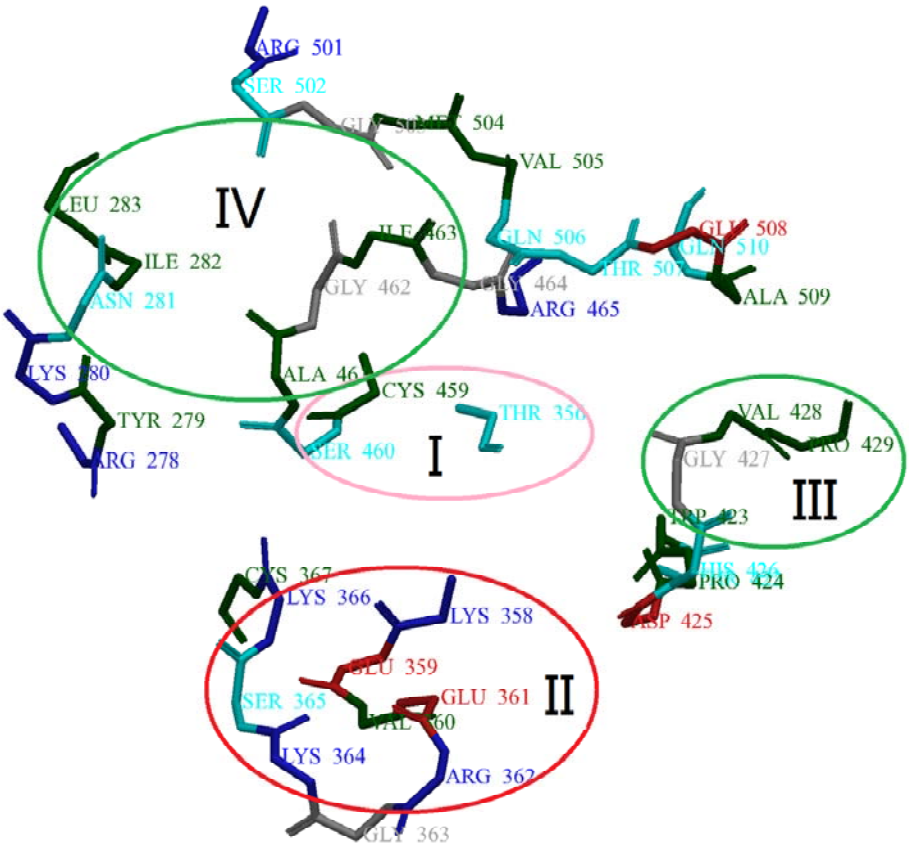
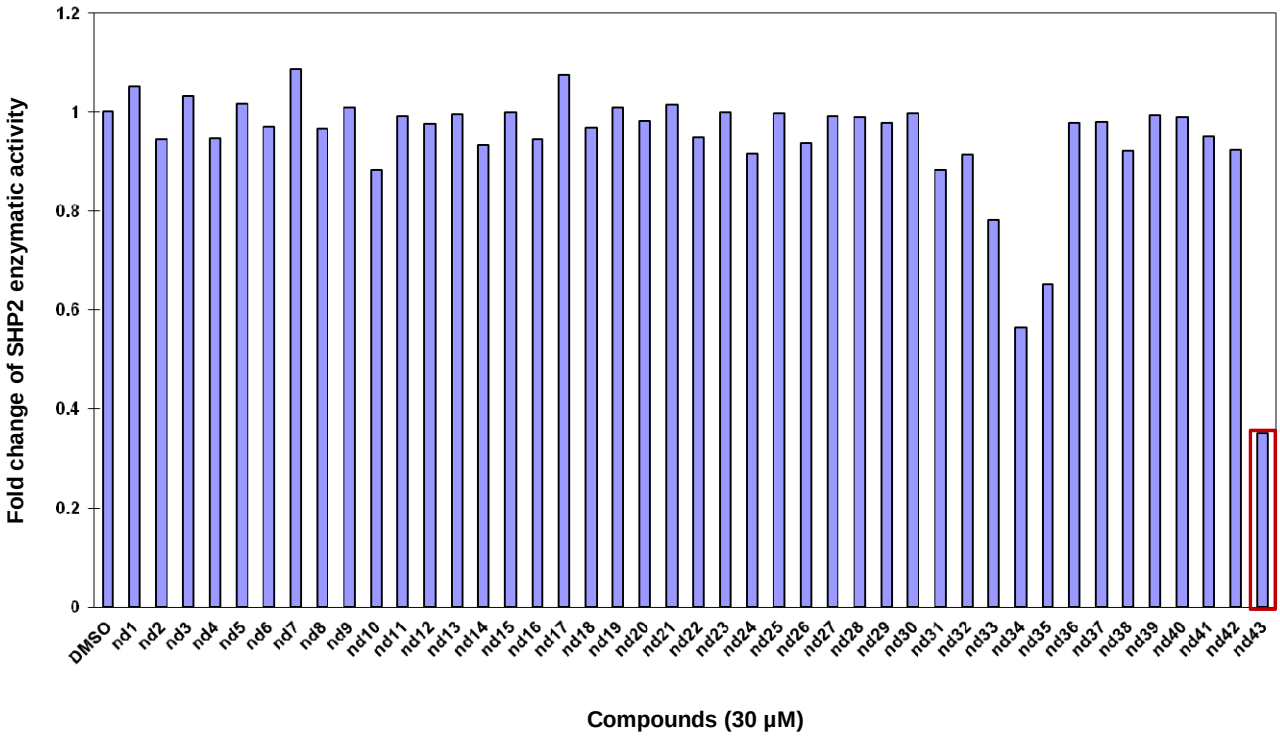
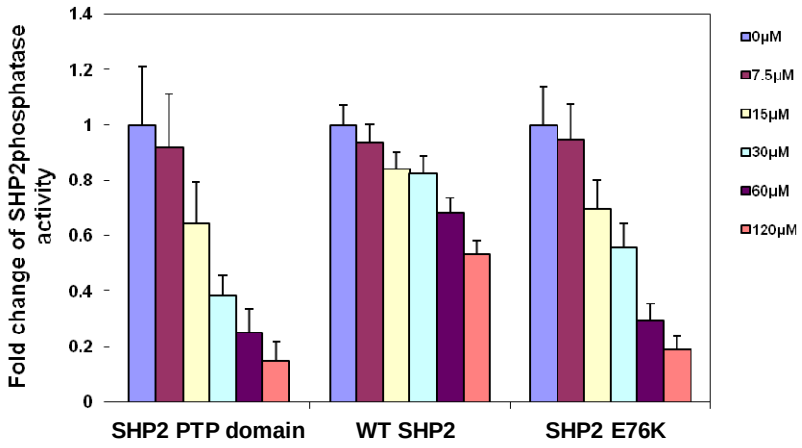


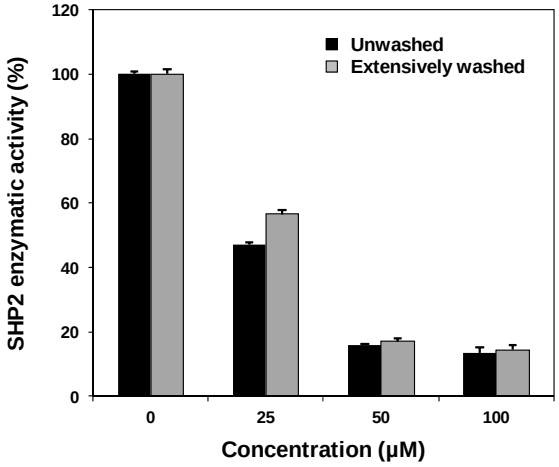
Supplementary Figure S1. Identification of a drug docking site in the SHP2 catalytic domain. 3D crystal structures of SHP2 and SHP1 were downloaded from Protein Data Bank (www.rcsb.org) with the ID codes 2SHP and 2B3O, respectively. I: Key residues for catalysis. II: Different edges between SHP2 and SHP1. III and IV: Hydrophobic zones. Residues are shown according to their properties. Basic, acidic, polar, and non-polar residues are colored in blue, red, shy blue, and green, respectively.



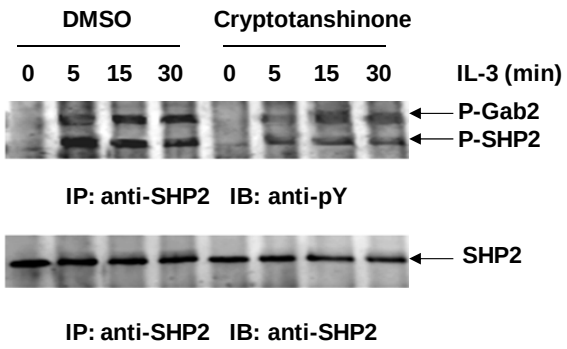
Supplementary Figure S2. Experimental screening of natural products indentified in the *in silico* database screening. Candidate compounds (30 μM) identified in the *in silico* database screening were subjected to the phosphatase assay using GST-SHP2 PTP domain as the enzyme and a phospho-EGFR peptide as the substrate. DMSO was used as a negative control. One active compound (Cryptotanshinone) marked in red was identified.



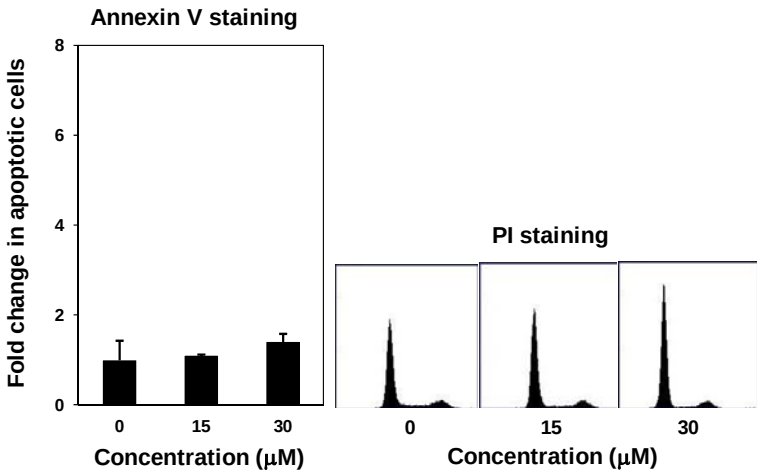
Supplementary Figure S3. Wild-type full length SHP2 is less sensitive to Cryptotanshinone than the SHP2 E76K mutant or the SHP2 PTP domain. Inhibitory effects of Cryptotanshinone at the indicated concentrations on the enzymatic activities of the SHP2 PTP domain, WT full length SHP2, and full length SHP2 E76K were determined using *p*NPP as the substrate. Experiments were repeated three times. Similar results were obtained in each. Data shown are mean±S.E.M. of triplicates from one experiment.



Supplementary Figure S4. Cryptotanshinone is an irreversible inhibitor of SHP2. GST-SHP2 PTP domain (0.5 μg) on glutathione agarose beads was incubated with Cryptotanshinone at the indicated concentrations at room temperature for 30 min. Beads were collected and washed with the phosphatase assay buffer three times. The phosphatase activity of GST-SHP2 PTP on the beads was determined by the phosphatase assay using a phospho-EGFR peptide as the substrate. Data are presented as mean±S.E.M. of three independent experiments.



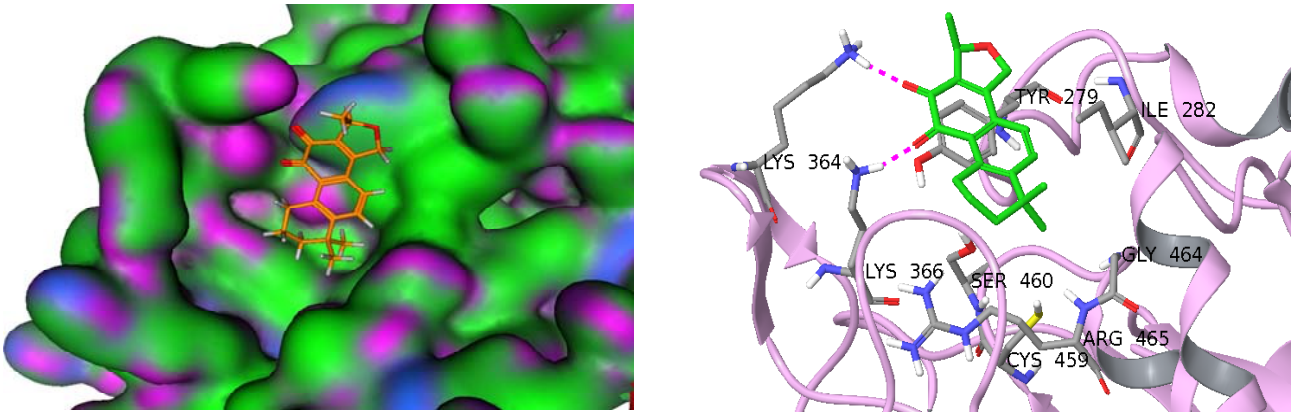
Supplementary Figure S5. Cryptotanshinone inhibits IL-3-induced Gab2-SHP2 interaction. Ba/F3 cells were deprived of IL-3 overnight. Cells were treated with Cryptotanshinone (30 μM) for 3 hours and then stimulated with IL-3 (1.0 ng/mL) for the indicated times. Whole cell lysates prepared were immunoprecipitated with anti-SHP2 antibody followed by anti-pY immunoblotting. Blots were stripped and reprobed with anti-SHP2 antibody. Experiments were repeated three times with similar results obtained in each. Representative blots from one experiment are shown.



Supplementary Figure S6. Cryptotanshinone induces cell cycle arrest in H661 cells with the *PTPN11*$^{N58S/+}$ mutation. Human lung cancer cell line H661 cells were incubated with Cryptotanshinone at the indicated concentrations. Twenty-four hours later, apoptotic cells were quantified by FACS using an Annexin V staining kit following the instructions provided by the manufacturer. Cell cycle changes were determined by propidium iodide staining followed by FACS analyses. Experiments were performed three times. Data are presented as mean±S.E.M. of three experiments. Cell cycle profiles shown on the right panel are representatives of three independent experiments.

A

B



Supplementary Figure S7. Predicted binding mode of Cryptotanshinone. (A) Computational binding mode of Cryptotanshinone with the SHP2 PTP domain (PDB code 2SHP). The H-bonding, hydrophobic, and mild polar surfaces are shown in purple, green, and blue, respectively. For Cryptotanshinone, carbon atoms are colored in orange, oxygen in red, hydrogen in white. (B) Predicted interactions between the amino acid residues of the PTP domain and Cryptotanshinone. Carbon atoms are colored in green. Oxygen atoms are shown in red. The H-bonds formed between Cryptotanshinone and the protein, via Lys364 and Lys366, are shown in purple dotted lines. The benzene ring structure of Cryptotanshinone is located toward Tyr279.