Structural reorganization of SHP2 by oncogenic mutations and implications for oncoprotein resistance to allosteric inhibition

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Activating mutations in PTPN11, encoding the cytosolic protein tyrosine phosphatase SHP2, result in developmental disorders and act as oncogenic drivers in patients with hematologic cancers. The allosteric inhibitor SHP099 stabilizes the wild-type SHP2 enzyme in an autoinhibited conformation that is itself destabilized by oncogenic mutations. Here, we report the impact of the highly activated and most frequently observed mutation, E76K, on the structure of SHP2, and investigate the effect of E76K and other oncogenic mutations on allosteric inhibition by SHP099. SHP2E76K adopts an open conformation but can be restored to the closed, autoinhibited conformation, near-identical to the unoccupied wild-type enzyme, when complexed with SHP099. SHP099 inhibitory activity against oncogenic SHP2 variants in vitro and in cells scales inversely with the activating strength of the mutation, indicating that either oncoselective or vastly more potent inhibitors will be necessary to suppress oncogenic signaling by the most strongly activating SHP2 mutations in cancer.
The non-receptor protein tyrosine phosphatase SHP2, encoded by the PTPN11 proto-oncogene, is required for normal development and functions downstream of growth factor, cytokine, and adhesion receptors. SHP2 is ubiquitously expressed and required for sustained Ras-MAP kinase activation, and also modulates signaling through the NF-κB, JAK-STAT, PI3K-AKT, programmed cell death 1, and immune checkpoint (BTLA) pathways. Activating mutations of SHP2 cause developmental disorders, such as Noonan Syndrome, and occur frequently in patients with juvenile myelomonocytic leukemia (35%). Activating mutations are also observed recurrently in acute myeloid leukemia (5%) and at lower frequencies in other hematological cancers and solid tumors. Cancer-associated mutations of SHP2 also cause leukemia in mice, whereas genetic or chemical suppression of SHP2 has antitumor activity in a variety of cancer models.

SHP2 contains two tandem SH2 domains (N-SH2 and C-SH2), a catalytic protein tyrosine phosphatase (PTP) domain, and a C-terminal tail that has at least two phosphorylation sites. The X-ray structure of SHP2 reveals that in the basal state, this protein adopts a closed, autoinhibited conformation in which the N-SH2 domain engages the catalytic pocket of the PTP domain and sterically occludes the active site. Normalizing the binding of tyrosine-phosphorylated ligands to the SHP2 tandem SH2 domains is required to overcome autoinhibition, but oncogenic mutations of SHP2 destabilize the autoinhibited conformation and lead to enhanced basal activity in the absence of tyrosine-phosphorylated ligand stimulation.

Allosteric modulators that stabilize the closed form of SHP2 have been recently reported. This class of allosteric inhibitors was designed to stabilize the autoinhibited state of the enzyme by acting as a molecular glue between the N-SH2 domain and the catalytic domain. One such compound, SHP099, binds to wild-type SHP2 with nanomolar affinity in biochemical assays, and exhibits antiproliferative activity in cancer cell lines that are dependent on receptor tyrosine kinases and wild-type SHP2. It remains unclear, however, whether SHP2 activating mutations are amenable to allosteric inhibition by compounds such as SHP099, and if so, what range of mutations are susceptible.

Here, we investigate the impact of oncogenic mutations on the structure of SHP2 and on allosteric inhibition by SHP099 in biochemical and cellular assays. We report an open-state structure of a SHP2 variant that bears a potent activating mutation, E76K, which induces a dramatic domain reorganization to expose the active site and eliminates the binding pocket for the allosteric inhibitor SHP099. Although the E76K mutation reduces the inhibitory potency of SHP099 for SHP2 by more than 100-fold, binding of SHP099 to SHP2E76K can revert the structure of this variant to its autoinhibited conformation. More generally, although a broad range of SHP2 oncogenic mutations can be inhibited by SHP099 in assays using the purified enzyme, the potency of inhibition scales inversely with the basal phosphatase activity of each variant, and in cells, the more active SHP2 oncogenes display resistance to allosteric inhibition. These data show that oncoselective SHP2 inhibitors, or vastly more potent allosteric inhibitors, will be necessary to suppress the aberrant signaling that results from strongly activating SHP2 mutations in cancer.

Results

Structure of SHP2E76K in an open conformation. The autoinhibited conformation of SHP2 (PDB:2SHP) is stabilized by interactions between residues of the N-SH2 domain and regions of the phosphatase (PTP) domain that mask the catalytic pocket. Although amino acids of the SHP2 C-SH2 domain do not directly interact with either the N-SH2 or PTP domains, the orientation of the C-SH2 domain in the autoinhibited conformation of SHP2 is stabilized through interactions of the unstructured loop between N-SH2 and C-SH2 domains with N-SH2 and PTP domains and of the linker between C-SH2 and PTP domains with the PTP domain.

To investigate how the most frequently observed SHP2 oncogenic mutation, E76K, affects the structure of SHP2, we resolved the X-ray structure of near full-length SHP2E76K (1–525) to 2.6 Å (Fig 1b and Supplementary Table 1). We found that, in comparison with the basal conformation of SHP2WT, the C-SH2 domain of SHP2E76K undergoes a 120-degree rotation with respect to the adjacent PTP domain, which fully exposes the PTP active site and repositions the N-SH2 domain onto a PTP domain surface opposite the catalytic pocket. The swiveling of the C-SH2 domain thus acts as a pivot to move the N-SH2 domain across the entire body of the PTP domain from one side to the other in switching from closed to open conformations. In both the basal SHP2WT and open SHP2E76K structures, the phosphotyrosyl binding site of each SH2 domain remains accessible to solvent.

The N-SH2 domain of SHP2, which is stabilized primarily through interdomain interactions in SHP2WT, is in part fixed by crystal contacts in SHP2E76K. Specifically, contacts between the αB helix and a symmetry-related PTP domain stabilize the position of the N-SH2 domain in SHP2E76K in one of the two copies in the asymmetric unit. Nevertheless, there are interdomain contacts between the N-SH2 domain and the PTP domain that create a remarkably different N-SH2-PTP interdomain interaction surface in SHP2E76K from the N-SH2-PTP interface observed in the autoinhibited conformation of SHP2WT.

The buried surface area of the N-SH2 domain decreases from 1025 Å² in SHP2WT to 357 Å² in E76K, with no overlapping contact residues. In SHP2E76K, the N-SH2 N-terminal residues and N terminus of the αB helix contribute to stabilizing the position of the N-SH2 domain at the alternative PTP surface. Specific interactions include a salt-bridge between R4 of the N-SH2 domain and D485 of the PTP domain, and hydrophobic contacts between T73 and V484.

The remodeled interface between the C-SH2 domain and the PTP domain in the open conformation is striking. Key residues that stabilize the position of the C-SH2 domain with respect to the PTP domain in the open-state structure of E76K lie in the segment preceding the C-SH2 domain core, and in the linker between the C-SH2 domain and the PTP domain. These residues include S109-R111, which contact E225, R229, and E232, and amino acids Q214 through I221 of the linker connecting the C-SH2 and PTP domains, which place several residues in backbone and side-chain contact with PTP domain residues D487 through D489, as well as with E249. One hydrogen bond, between T219 and D489, is conserved in both basal wild-type and E76K open conformations of SHP2.

Remarkably, the open conformation of SHP2E76K also resembles the architecture of SHP1 (PDB:3PS5) in its open conformation. In the open state of SHP1, the C-SH2 domain also pivots 110-degrees relative to its position in the autoinhibited conformation of SHP1, which repositions the N-SH2 onto a surface of the SHP1 PTP domain similar in location to the N-SH2-PTP interface observed in the open conformation of SHP2E76K. In SHP1, however, the interactions between the N-SH2 and PTP domains are more extensive, burying 511 Å² of surface area, which is substantially more than the 357 Å² buried at this interface in the SHP2E76K structure.

SHP099 binding to SHP2E76K restores the closed conformation. To determine whether SHP099 could revert SHP2E76K to
the closed, autoinhibited conformation, we determined the structure of SHP2E76K bound to the small molecule inhibitor SHP099 to 2.8 Å (Supplementary Table I). Remarkably, when complexed with SHP099, SHP2E76K adopts an autoinhibited conformation like that of SHP2WT, with near-identical C-SH2 and N-SH2 domain orientations relative to the PTP domain (Fig. 1e). The interactions between SHP099 and SHP2E76K are similar to those seen in the structure of SHP099 bound to SHP2WT (PDB: 5EHR). However, at the N-SH2–PTP interface, the K76 side chain is not in contact with the PTP surface and is instead poised to interact with Q79, which stabilizes the position of K76 in the open state of SHP2E76K. Together, the X-ray structures of unliganded and SHP099-bound SHP2E76K show that the unoccupied enzyme adopts an open conformation that is similar to the open form of SHP1, and that binding of SHP099 reverts SHP2E76K to a closed conformation that is near-identical to the autoinhibited state of SHP2WT.

To test whether the X-ray structure of SHP2E76K in the crystal is representative of its conformation in solution, we performed small-angle X-ray scattering (SAXS) analysis of SHP2E76K and compared it with SAXS data acquired for SHP2WT. Fitting of the background-subtracted scattering profiles of each protein (Fig. 2a) to the pair distance distribution p(r) function revealed that both proteins adopt compact conformations, with radii of gyration (Rg) of 26.2 ± 0.2 Å for SHP2WT and 29.2 ± 0.3 Å for SHP2E76K (Fig. 2b and Supplementary Table 2). The tail of the SHP2E76K p(r) function displayed a shallower slope than that of SHP2WT, resulting in a calculated maximal end-to-end distance

**Fig. 1** The open conformation of SHP2 E76K is closed by SHP099. a Basal structure of autoinhibited SHP2WT (PDB:2SHP) with the N-SH2 domain displayed in green, C-SH2 in blue, and PTP in beige. b Structure of SHP2E76K (1–525) reveals a 120° rotation of the C-SH2 domain, relocation of the N-SH2 domain to a PTP surface opposite the active site, and a solvent-exposed catalytic pocket. Insets show select N-SH2–PTP interdomain contacts and interactions between the C-SH2 and PTP domains. c Cartoon illustrating the domain movements that occur in SHP2E76K upon adopting the open conformation. E128 and D94 are chosen as arbitrary reference points to illustrate the effect of the 120° rotation. d Open conformation of SHP1 (PDB:3PP5) is similar to that of SHP2E76K. e Interaction of SHP099 with SHP2E76K rescues the autoinhibited conformation. Insets show select interactions of SHP099 with SHP2E76K and SHP2WT, in addition to differential orientations of residue 76 in SHP2E76K bound to SHP099 and in SHP2WT bound to SHP099 (White, PDB:5EHR)
(D\text{max}) of 100 ± 4 Å, whereas the D\text{max} for SHP2\text{WT} was 83 ± 3 Å.

Kratky analysis of the SAXS data for both proteins indicates that they are both well-folded and monodisperse in solution (Fig. 2c).

To assess whether the SAXS-derived particle dimensions of SHP2\text{E76K} are compatible with the conformation of SHP2\text{E76K} seen in the X-ray structure, we compared SAXS envelopes of SHP2\text{WT} and SHP2\text{E76K} with their respective X-ray structures. The SAXS-determined SHP2\text{WT} particle envelope agrees with the X-ray structure of SHP2 WT (PDB:2SHP) in its closed conformation (NSD: 0.94, Chi: 0.45, respectively (Fig. 2d)). Likewise, the X-ray structure of SHP2\text{E76K} fits the SHP2\text{E76K} SAXS envelope particularly well (NSD: 0.99, Chi: 0.77; Fig. 2e). Taken together, these results provide additional evidence that SHP2\text{E76K} adopts a solution conformation consistent with the open conformation seen crystallographically.

SHP2 oncprotein sensitivity to allosteric modulators. To investigate the biochemical effects of oncogenic mutations of SHP2 on allosteric activation and inhibition, we purified a series of cancer-associated SHP2 mutants that exhibit a spectrum of basal phosphatase activity. The oncogenic mutations typically reside in the N-SH2 or PTP domains of the phosphatase, and map almost exclusively to the interface between these domains in the autoinhibited structure of SHP2 (Fig. 3a). Using the fluorogenic substrate 6,8-Difluoro-4-Methylumbelliferyl Phosphate (DiFMUP), the basal activity of the SHP2 variants investigated here increases from SHP2\text{WT} < SHP2\text{F285S} < SHP2\text{G60V} < SHP2\text{S502P} < SHP2\text{E76K}, where SHP2\text{E76K} displays activity similar to the isolated PTP domain (SHP2\text{PTP}, Fig. 3b). To explore how SHP2 cancer mutations influence stimulation of the phosphatase by phospholigands, we titrated SHP2 variants with a synthetic bisphosphorylated peptide derived from IRS-1 (p-IRS-1), which stimulates phosphatase activity by engaging both the N-SH2 and C-SH2 domains, and quantified the effect on DiFMUP dephosphorylation as a function of added p-IRS-1 concentration (Fig. 3c). This analysis shows that the oncogenic mutations of SHP2 tested here do not detectably alter the maximal catalytic activity of the protein as observed in the presence of a saturating concentration of p-IRS-1, but rather increase both basal phosphatase activity and the sensitivity to phospholigand stimulation (Fig. 3d). Specifically, the cancer-associated SHP2 mutants reduce the concentration of p-IRS-1 required to stimulate the phosphatase activity of SHP2 by one to two orders of magnitude (the p-IRS-1 AC50 value for stimulation), except in the case of the most active mutant SHP2\text{E76K}, which is essentially fully activated in the absence of p-IRS-1 and thus insensitive to further stimulation (Fig. 3d).

To investigate how the various oncogenic SHP2 mutations impact allosteric inhibition of the phosphatase by SHP099, we titrated SHP099 against SHP2 proteins in the presence and absence of p-IRS-1 and quantified the phosphatase activity of the different enzymes using DiFMUP (Fig. 4a, b). We found that...
under basal conditions, SHP2 cancer mutations weaken the observed potency of SHP099 when compared with SHP2WT, and that the inhibitory potency of SHP099 generally scales inversely with the basal phosphatase activity of the cancer mutant (Fig. 4 and Supplementary Table 3). For the most active mutant SHP2E76K, we extended the dose–response curve to a higher SHP099 concentration in order to deduce its IC50 of 34 ± 6 μM in the absence of p-IRS-1 (Supplementary Fig. 1). As anticipated, the presence of added p-IRS-1 decreases the inhibitory potency of SHP099 for all forms of SHP2, with the relationship between p-IRS-1 and SHP099 fitting a competitive binding model (Fig. 4c). The IC50 of SHP099 for SHP2WT, for example, increases from 92 μM (at a saturating concentration of 10 μM p-IRS-1 peptide) to 1.7 μM (at a saturating concentration of 10 μM p-IRS-1 peptide). Other SHP2 cancer mutants show a similar trend in the presence of saturating (10 μM) p-IRS-1. Data points are presented as mean ± standard deviation (n = 3).

**Sensitivity of SHP2 signaling to SHP099 in cellular assays.** Having defined the effects of oncogenic SHP2 mutations on SHP099 allosteric inhibition of the purified proteins, we next sought to assess the sensitivity of this spectrum of cancer mutants to modulation of signaling by SHP099 in cells. For this purpose, we prepared a SHP2-deficient cell line using the CRISPR-Cas9 system (Fig. 5a, b), and then added either SHP2WT or different cancer variants for evaluation, measuring phosphorylated-Erk (pErk) levels as a readout of SHP099 inhibitory activity. We performed the knockout in U2OS cells, because this cell line induces pERK in response to EGF stimulation, which is attenuated in the presence of SHP099 (Supplementary Fig. 3), and because U2OS cells can tolerate SHP2 knockout. Although SHP099 treatment dramatically reduces pErk levels of wild-type U2OS cells, as assessed by western blot (Fig. 5c), pErk levels in PTPN11-null U2OS cells are unaffected by SHP099 treatment (Fig. 5d). Reintroduction of full-length SHP2WT into PTPN11-null U2OS cells by transfection results in expression of SHP2 in amounts about five-fold greater than in parental U2OS cells, as assessed by western blot (Fig. 5b). In PTPN11-null U2OS cells that re-express SHP2WT, pErk sensitivity to SHP099 is partially restored (Fig. 5e; we attribute the incomplete ~ 65% reduction of pErk levels by SHP099 in SHP2WT-rescued cells to a rewiring of SHP2 signaling that occurs upon SHP2 knockout, as evidenced by increased basal pErk levels in the null cells compared with the parental line). Quantification of the western blot data gives an IC50 value for pErk reduction by SHP099 in the PTPN11-null cells re-expressing SHP2WT (4 ± 2 μM) in line with the IC50 observed for wild-type U2OS cells (2 ± 1 μM; Fig. 5f). We then probed the effects of oncogenic SHP2 mutants on the sensitivity to SHP099 in the same assay. SHP2 cancer mutants (SHP2G60V, SHP2D61V, SHP2E76K, SHP2F285S, and SHP2S502F) introduced into PTPN11-null U2OS cells were expressed in amounts comparable to the reintroduced SHP2WT in the SHP2-null cells (Fig. 6a). The expression of these oncogenic variants in PTPN11-null U2OS cells is associated with pErk levels approximately five-fold greater than in parental U2OS cells (Fig. 6b), indicating that these mutants strongly stimulate this pathway when transfectedd
into the null cell line. SHP099 treatment reduces pErk levels in U2OS cells harboring the weakly stimulating SHP2F285S enzyme (Fig. 6c), with an IC_{50} for pErk reduction approximately four-fold greater (8 ± 2 μM) than in SHP2WT cells (Fig. 6d); in contrast, SHP2S502P displays little, if any, loss of pErk at 50 μM SHP099, and SHP099 does not significantly inhibit Erk phosphorylation in the other cancer mutants investigated. We also enforced expression of wild-type and oncogenic forms of SHP2 in the parental U2OS cells and observed qualitatively similar findings (Supplementary Fig. 4). These results indicate that oncoproteins with weakly activating mutations, such as F285S, remain sensitive to enzyme inhibition in cells, whereas more strongly activating mutations are effectively resistant to allosteric inhibition by SHP099.

**Discussion**

There is great interest not only in understanding how frequently observed cancer mutations in oncogenes and tumor suppressors impact protein structure and function, but also in deducing how such mutations affect therapies that target the wild-type protein. Here, we have pursued a mechanistic understanding of how activating mutations affect the structure of the protein phosphatase SHP2 and its sensitivity to the wild-type directed allosteric inhibitor SHP099. Strikingly, the most frequently occurring SHP2 cancer mutation, a potent E76K substitution also found in Noonan syndrome and cancer, lead to increased phosphatase activity. The open state of SHP2E76K involves a 120-degree pivot of the C-SH2 domain, relocalization of the N-SH2 domain to an alternative PTP domain interaction surface, and full exposure of the phosphatase active site. The contacts that stabilize this open C-SH2 domain orientation suggest that, in the absence of critical interactions at the N-SH2•PTP autoinhibition interface, this position is the preferred orientation of the C-SH2 domain, and likely also represents an accessible open conformation of wild-type SHP2 upon activation by phosphotyrosine-containing peptide sequences, as opposed to a neomorphic conformation resulting from the oncogenic E76K mutation. The discrete conformation of SHP2E76K in its open state was unexpected, but the remarkable agreement between the SHP2E76K crystal structure, SALS-determined solution particle dimensions, and the structure of the related phosphatase SHP1 in its open state further underscore the relevance of the open state structure.

Recently, allosteric inhibitors of SHP2 have been discovered that biochemically suppress phosphatase activation in the presence of stimulatory phospholigands and weakly activating cancer mutations. Although such molecules have been demonstrated to reduce the viability of RTK-driven tumor cells through inhibition of MAPK signaling in cells that have normal SHP2 alleles, we lack an understanding of whether SHP2 mutations observed in patients with PTPN11-mutated malignancies can be effectively targeted by currently available allosteric inhibitors.

Strikingly, binding of the SHP2 allosteric inhibitor SHP099 to SHP2E76K can revert SHP2E76K to the autoinhibited conformation seen in the basal state of SHP2WT. We found, however, that the efficacy and potency of SHP099 inhibition decreases as the amount of stimulatory phospholigand or as the strength of the activating mutation increases. SHP099 inhibition of the most...
active oncogenic SHP2 mutants investigated here, SHP2-D61V and SHP2-E76K, are orders of magnitude weaker than inhibition of SHP2WT, and the responsiveness of the mutants tested using purified protein is mirrored in cell lines engineered to express the same spectrum of mutated SHP2 proteins. Thus, although it is possible to revert the most potent activating mutation back to the closed conformation with SHP099 in vitro, cell lines expressing strongly activating mutations are effectively resistant to SHP099, likely because stabilization of the open conformation by phosphotyrosine ligands further increases the favorability of the open state in cells.

Because nearly half of patients with *PTPN11*-mutated cancers bear strongly activating mutations of E76, D61, G60, and S502, an allosteric inhibitor would need to exhibit at least two additional orders of magnitude greater potency than SHP099 and a longer target residence time to achieve successful suppression of SHP2 activity of tumors bearing these mutations in a clinical setting. Whereas strongly activating mutations of SHP2 confer resistance to allosteric inhibition, orthosteric SHP2 inhibitors, in principle, may exhibit more effective oncogene selectivity because their mechanism of action targets the open conformation favored by cancer-associated mutations. The primary challenge with orthosteric inhibitors is to achieve sufficient selectivity for SHP2 over other cellular phosphatases, which may be further thwarted by the SHP2 cancer mutations that localize to residues near the active site, such as S502, G503, and Q510. One orthosteric inhibitor, called 11-a 1, is reported to have fivefold or greater selectivity for SHP2 over related protein tyrosine phosphatases and to show promise in a melanoma model. However, another recent study has called into question the specificity of such active site-directed inhibitors in the cellular context. A combination of potent allosteric inhibitors that target distinct pockets may be yet another alternative strategy for inhibiting active SHP2 oncogenic mutants. Finally, the open state structure suggests an experimental path for discovery of allosteric or active-state modulators that stabilize conformations of SHP2 other than its basal, autoinhibited state in strongly activated mutants.

**Methods**

**Materials.** Difluoromethyluracil (D22065) and 6,8-difluoro-7-hydroxy-4-methylcoumarin (Difluo; D6566) were purchased from Life Technologies, Tris(2-carboxyethyl)phosphine hydrochloride from PierceTM (20490), potassium bisperoxo(1,10-phenanthroline) oxovanadate (bpV(Phen)) from Enzo Life Sciences (ALX-270-204) and phosphorylated peptides from AnaSpec. Unless otherwise stated, all reagents were obtained from commercial sources and used without further purification.

**SAXS.** Size-exclusion chromatography (SEC)–SAXS experiments were performed at BioCAT (beamline 18-ID; Advanced Photon Source, Argonne National Laboratory). The setup included a focused 12-keV (1.03 Å) X-ray beam, a 1.5-mm quartz capillary sample cell, a sample-to-detector distance of ~2.5 m, and a...
Oncogenic mutants of SHP2 negatively impact allosteric inhibition in SHP2-deficient U2OS cells. 

**Fig. 6**

**a** Western blot quantification of SHP2 expression levels in wild-type U2OS cells (U2OS) and in SHP2-deficient U2OS cells (PTPN11-null) in the presence and absence of expression of SHP2 oncogenic variants. 

**b** Western blot analysis showing relative pErk levels in wild-type, SHP2-deficient, and SHP2-rescued U2OS cells. 

**c** Western blot determination of pErk levels in SHP2-deficient U2OS cells expressing SHP2WT and oncogenic mutants upon titration of SHP099. Data points are presented as mean ± standard error of the mean.

**d** Quantification of pErk levels in SHP2-deficient U2OS cells expressing SHP2WT and oncogenic mutants upon titration of SHP099. Data points are presented as mean ± standard error of the mean.

Quantification of basal SHP2 phosphatase activity. SHP2 proteins were expressed and basal activity was measured as described13. In brief, 2.5 nM SHP2WT, 0.5 nM SHP2F285S, 0.1 nM SHP2G60V, 0.05 nM SHP2S502P, and SHP2D61V, and 0.025 nM SHP2E76K and SHP2F285S were prepared in 60 mM Hepes pH 7.2, 75 mM KCl, 75 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.05% Tween-20, and 1 mM tris(2-carboxyethyl)phosphine (TCEP) (buffer 1), and added to a linear titration of DiFMUP (4 μM to 1 fM). DiFMUP dephosphorylation was measured at ex. 358, em. 450 nm on a Spectramax M5, and raw velocity data was converted to product 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU)/time/through preparing a standard curve of DiFMU/DiFMUP. To allow direct comparison between enzyme trajectories, velocity data were normalized to enzyme concentration to yield relative velocity, which was then fit to the following substrate inhibition equation (Eq. (1)) to extrapolate kinetic parameters:

$$ V = V_{\text{max}} - \frac{[S]}{K_S + [S](1 + [S]/K_I))} $$

Where $V$ is enzyme velocity, $V_{\text{max}}$ is maximal enzyme activity, $K_S$ is the Michaelis–Menten constant, [S] is substrate concentration, and $K_I$ is the substrate dissociation constant. In vitro SHP2 phosphatase assays were performed in triplicate on the same day.

**SHP2 phosphatase activity upon p-IRS-1 peptide stimulation.** Wild-type and mutant SHP2 proteins were diluted to 0.1 nM and treated with either varying concentrations of diphosphorylated IRS-1 peptide (pY1172-Peg-PY1222, SLNY(p) IDLDLVK-dPEG8-LSTY(p)ASINFQK), or for maximal activation, 10 μM pY1172-Peg-PY1222. Phosphatase activity was measured through the dephosphorylation DiFMUP (200 μM, ex. 385, em. 450 nm) on a Spectramax M5 plate reader and converted to product (DiFMU)/time/through preparing a standard curve of DiFMU/DiFMUP. Velocity data were fit to the following three-parameter dose–response curve (Eq. (2)) to extrapolate EC50 values:

$$ V = \frac{\text{Max} - \text{Basal}}{1 + 10^{(\text{LogEC}_{50} - [S])}} $$

Where $V$ is the response, Max is the maximal enzyme activity, Basal is the response, and EC50 is the effective concentration.
Where $V$ represents enzyme velocity, $B_s$ is basal response, $X$ is maximal response, and $E_{C_50}$ is the substrate concentration that yields response half way between basal and maximal.

The influence of SHP2 cancer mutations on SHP099 efficacy. Wild-type and mutant SHP2 proteins were diluted to 0.1 nM in buffer 1, in the presence of SHP099, ranging in concentration from 100 nM to 5 nM. To which, bis-tyrrosinephosphorylated peptide py1172-Peg-PY1222 was either added at 10 nM or titrated, from 10 nM to 1 nM, followed by 100 μM DMAP. The reaction was then allowed to proceed for 30 minutes at room temperature, quenched with bvp(Phen) and assayed on a PerkinElmer EnVision™ plate reader. For visualization, raw data were converted to percent effect (Eq. (3)) and fit to a variable slope four-parameter inhibitor response curve (Eq. (4)) to extrapolate SHP099 IC50 values.

Percent Effect = \( \frac{x - \text{min}}{\text{max} - \text{min}} \times 100\% \)  

Where $x$ is the sample value (RFU), min is the RFU observed for SHP2WT in the presence of 100 μM SHP099 and absence of py1172-Peg-PY1222, and max is the maximum RFU observed for respective SHP2 protein in the absence of SHP099 and presence of 10 μM py1172-Peg-PY1222, after reacting with 100 μM DFMUP for 30 min at room temperature, and max is the maximum RFU observed.

Percent Effect = \[ \frac{\text{max} - \text{min}}{1 + \text{EC}_{50} - \text{min}} \times \text{time} \]

Where $x$ and min are the respective maximum and minimum values of each dose–response curve, IC50 is the SHP099 concentration that gives response halfway between max and min, and hillshape describes the steepness of each curve. In vitro SHP099 dose responses were performed in high throughput, with two technical replicas, on the same day.

Design of SHP2 expression plasmids. Mammalian SHP2 expression plasmids were designed from pCMV-SHP2 WT, which was provided as a gift from Ben Neel et al. In brief, C.9′-CACCGGATTACTATGACCTGTATGG-3′ primers were designed from pCMV-SHP2 WT, which was provided as a gift from Ben Neel. Design of SHP2 expression plasmids

Western blot pErK quantification in U2OS/PTPN11-null cells. 1.0 Å 106

PTPN11+/−/− U2OS (Parental U2OS; ATCC® HTB-96®) cells were plated in six-well tissue culture-treated plates (Corning® LS3516) in complete growth media two days prior to experiment or 1.0 × 106 U2OS cells were plated 1 day prior to SHP099 addition and allowed to adhere. For PTPN11+/−/− U2OS cells, one day prior to addition of SHP099, cells were transfected with respective CMV-driven SHP2 plasmids using manufacturer-recommended FuGENE®HD methodologies, with 0.6 μg DNA and 2.4 μL FuGENE®HD per well. On the day of experiment, media was aspirated and replaced with 3 mL media containing either SHP099 at respective concentration or 0.03% dimethyl sulfoxide, and cells were placed at 37 °C with 5% CO2 for 2 h. After SHP099 treatment, cells were lysed with cold RIPA buffer (80 μL) containing Complete® EDTA-free protease inhibitor and frozen using a microfluidizer, followed by ultracentrifugation. The supernatant was loaded onto a HiTrap HP chelating column in 50 mM Tris-HCl pH 8.5 and 1 mM TCEP and to a HiTrap Q Fast-Flow column equilibrated with 20 mM Tris pH 8.5, 50 mM NaCl, and 1 mM TCEP. The protein was eluted with a 10-column volume gradient, from 50 to 500 mM NaCl. Fractions containing SHP2 were pooled, concentrated, and loaded onto a HiLoad Superdex200 26/60 column in 50 mM Tris-HCl pH 8.5, 150 mM NaCl, 1 mM TCEP, and 5% (v/v) glycerol. SHP2E76K was concentrated to 10 mg/ml for use in crystallization.

Expression and purification of SHP2E76K. The gene encoding human SHP2 from residues Met1-Leu525 was inserted into a pET30 vector, and a sequence encoding a 6× histidine tag followed by a tobacco etch virus (TEV) protease cleavage site was added 5′ to the SHP2 gene sequence. The E76K mutation was introduced using an Agilent QuickChange® Site-Directed Mutagenesis Kit. The resulting construct was transformed into BL21 Star® (DE3) cells and grown at 37 °C in Terrific Broth containing 100 μg/ml kanamycin. At an OD600 of 4.0, SHP2 expression was induced using 1 mM IPTG. Cells were harvested following overnight growth at 18 °C. Cell pellets were resuspended in lysis buffer containing 50 mM Tris-HCl pH 8.5, 2.5 mM imidazole, 500 mM NaCl, 2.5 mM MgCl2, 1 mM TCEP, 1 μg/ml DNase1, and Complete® EDTA-free protease inhibitor and frozen using a microfluidizer, followed by ultracentrifugation. The supernatant was loaded onto a HiTrap Trap Heparin chelating column in 50 mM Tris-HC1, 25 mM Imidazole, 500 mM NaCl, 1 mM TCEP, and SHP2E76K was eluted with the addition of 250 mM imidazole. The N-terminal histidine tag was removed with an overnight incubation with TEV protease at 4 °C. The protein was subsequently diluted to 50 mM NaCl with 20 mM Tris-HCl pH 8.5 and 1 mM TCEP and applied to a HiTrap Q Fast-Flow column equilibrated with 20 mM Tris pH 8.5, 50 mM NaCl, and 1 mM TCEP. The protein was eluted with a 10-column volume gradient, from 50 to 500 mM NaCl. Fractions containing SHP2 were pooled, concentrated, and loaded onto a HiLoad Superdex200 26/60 column in 50 mM Tris-HCl pH 8.5, 150 mM NaCl, 1 mM TCEP, and 5% (v/v) glycerol. SHP2E76K was concentrated to 10 mg/ml for use in crystallization.

Crystalization and structure determination. The sitting-drop vapor diffusion method was used for crystallization of both SHP2E76K apo and SHP2E76K in complex with SHP099. SHP2E76K apo was crystallized using a 1:1 volume of protein and well solution (0.1 M MES pH 6.5, 12% polyethylene glycol (PEG) 20,000, and 5% sucrose). Crystals were cryoprotected using the crystallization solution with the addition of 20% glycerol followed by flash freezing directly into liquid nitrogen. SHP2E76K in complex with SHP099 was co-crystallized using a 1:1 volume of protein + 2 mM compound and well solution (0.1 M Tris pH 8.5, 30% PEG 4000, and 0.2 M LiSO4). Crystals were cryoprotected using the crystallization solution + 1 mM compound with the addition of 20% glycerol followed by flash freezing directly into liquid nitrogen.

Diffraction data for both SHP2E76K apo and SHP2E76K in complex with SHP099 were collected on a Dectris Pilatus 6 M Detector at beamline 17ID (IMCA-CAT) at the Advanced Photon Source at Argonne National Laboratories. The data were measured from a single crystal maintained at 100 K and exposed to a wavelength of 1 Å, and the reflections were indexed, integrated, and scaled using XDS27. The spacegroup of SHP2E76K apo was C2221 with two molecules in the asymmetric unit. The structure was determined by molecular replacement using Phaser28, with the structure of SHP2PT (PDB: 2SHP) used as a search model. All solvent molecules were removed and the model was divided into its individual domains of N-SH2 (4–104), C-SH2 (112–215) for chain A, and PTP (219–525). The final model was built in the Coot molecular graphics application29 and refined using Buster (Global Phasing, Ltd.30). The spacegroup of SHP2E76K in complex with SHP099 was P21, with two molecules in the asymmetric unit. The structure was determined with Fourier methods, using the SHP2WT structure (PDB: 2SHP) with all solvent molecules removed. The final model was built in the Coot molecular graphics application and refined using Buster. Data collection and refinement statistics are shown in Supplementary Table 1.

Data availability Coordinates and structure factors for the SHP2E76K apoprotein structure and for the SHP099-bound structure of SHP2E76K are deposited in the protein data bank under PDB ID codes 6CRF and 6CGR, respectively. All other source data are available from the corresponding authors on reasonable request and/or are included with the manuscript (as figure source data or Supplementary Information).

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S.C.B., M.G.A., M.J.M., R.C., M.M., and T.S. initiated the project. J.R.L., M.F., V.V.,
and P.W. performed biochemical and cell-based assays. M.F. crystallized the E76K mutant,
acquired diffraction data, and solved the structure. J.R.L. and S.C.B. wrote the manuscript
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