**Corrections**

**BIOCHEMISTRY**

The undersigned authors wish to note, “The KefFC system of *E. coli* is maintained in an inactive state by the binding of glutathione (GSH) and is activated by the formation of GSH adducts (GSX), particularly those with bulky substituents. We described two crystal structures with density present in the ligand-binding domain that we interpreted as GSH and GSX. Recently, an independent, experienced crystallographer, who had viewed the structures from our study in a different context, made representations to us that cast doubt on position of the succinimido ring of GSX. We have further reviewed the density maps with the aid of an experienced crystallographer. As a consequence, we believe it is important to draw this altered interpretation of the crystal structures to the attention of readers. In both structures, the density for the backbone of GSH is clear and allows unequivocal assignment of the position of the tripeptide. In PDB coordinate 3L9X, the density for the succinimido ring is very weak, making interpretation very speculative and the assignment rests on the identity of the ligand added to the crystallization mixture, for which there are two diastereomers in the solution—a possibility that provides some basis for weakening the density. However, in 3L9W there are two anomalies that affect the interpretation of the bound ligand. First, there is no density for the carbon atom attached to the sulfur of GSH and second, there is extra density adjacent to the position of sulfur that could be modelled as a constrained succinimido ring. However, this density could also be water or any other molecule that is trapped in the structure. Thus, while there is good evidence for the peptide, the evidence that it is in the GSH form is uncertain.

“There are no new data on either the structures or on the gating mechanism. However, we believe that we should be cautious in interpreting the structural data and that the field in general should be made aware of the alternative views of the electron density data. Note that the mutagenesis and spectroscopic data that were presented in the original manuscript are not affected by this alternative interpretation.”

Tarmo P. Roosild and Samantha Castronovo have decided not to sign this statement.

**NEUROSCIENCE**

The authors note that the following grant should be added to the Acknowledgments: “NIH Grant AG002132.”

**PHARMACOLOGY**

The authors note that the following statement should be added to the Acknowledgments: “We want to thank the generosity of Drs. Marcia I. Dawson and Zebin Xua (Sanford-Burnham Medical Research Institute) for providing us with 3-CI-AHPC and for their discussion on its use.”

**PHYSIOLOGY**

The authors note “The method used for exogenous expression of CαV1.2 channels in ref. 32 was incorrectly described as ‘viral transduction’ in the text. In fact, Yang et al. created transgenic mice with inducible, cardiomyocyte-specific expression of exogenous CαV1.2 channels regulated by a tetracycline-inducible promoter. When crossed with a transgenic mouse line expressing doxycycline-regulated reverse transcriptional activator under control of the α-myosin heavy chain promoter, the resulting double transgenic offspring expressed exogenous CαV1.2 channels in their cardiac myocytes after treatment with doxycycline. The authors regret the error in describing these methods.”

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Nuclear receptors form a family of ligand-regulated transcription factors that govern a wide array of cellular activities, including cell proliferation, differentiation, metabolism, and death (1). The nuclear receptor family also contains many orphan members for which no ligand is known (1). Although some of these orphan nuclear receptors function as transcriptional activators, others serve primarily as transcriptional repressors (2). The molecular basis for ligand-regulated transcription by nuclear receptors has been intensely investigated over the past three decades, and structural studies have revealed that ligand-activated receptors use the C-terminal activation function-2 (AF-2) helix (also called helix H12) of the ligand-binding domain (LBD) to recruit coactivators that contain LXXLL motifs (3–9). Antagonist binding destabilizes the AF-2 helix from the canonical LBD fold, thus opening up an extended groove for interactions with the longer LXXLXXX (L/I) motifs present in nuclear receptor corepressors such as NCoR and SMRT (10). Although these studies have provided a clear molecular basis for how ligands regulate AF-2 helix-dependent transcriptional activity, the molecular basis for ligand-independent repression by orphan nuclear receptors remains poorly understood.

Small heterodimer partner (SHP) is an orphan nuclear receptor that functions primarily as a transcriptional repressor. This orphan nuclear receptor causes the release of coactivators, thus decreasing gene activation. In the second step, SHP actively recruits corepressors to inhibit gene transcription. Identification of SHP-interacting corepressors has been a subject of intense study in recent years (19–25). As a result, a number of proteins have been isolated that bind to SHP in vitro or in vivo assays, one of which is E1A-like inhibitor of differentiation (EID1) (24). EID1 was first cloned as an interacting protein for the retinoic acid receptor α (26). EID1 inhibited skeletal muscle cell differentiation by repressing muscle-specific gene expression. This was due to its ability to bind to p300/CREB coactivators and inhibit their histone acetyltransferase (HAT) activity (27). Later, E1D1 was found to also interact with SHP (24). Because of its intrinsic transcriptional repression activity, EID1 has been suggested to be a SHP corepressor in several studies (24, 28–30).

Although the detailed molecular basis underlying SHP’s inhibitory function is still under intense investigation, the physiological roles of SHP in regulating bile acid and cholesterol homeostasis in liver are well characterized (13, 14, 31, 32). SHP-mediated repression is a key component of a feedback loop that represses the expression of genes that encode several key hydroxylase enzymes involved in bile acid biosynthesis, such as CYP7A1 and CYP8B1, as well as SHP itself (13, 14). Accordingly, CYP7A1 expression and bile acid synthesis are increased in SHP knockout mice (31, 32). Interestingly, the repressor activity...
of SHP is itself tightly regulated. SHP inhibition of CYP7A1 in liver is regulated by fibroblast growth factor 15 (FGF15), which is a hormone secreted from the small intestine (33). SHP-mediated repression is abolished in mice lacking FGF15, leading to abnormally high levels of CYP7A1 expression and fecal bile acid excretion (33). A number of retinoid-like compounds have been shown to bind to SHP and enhance its repression of CYP7A1 and CYP8B1 in liver cells (34–36). These findings underscore the importance of understanding the functional and structural basis for SHP’s inhibitory function, which could serve as a drug target for treating metabolic diseases arising from bile acid and cholesterol imbalances.

In this paper, we present a 2.8-Å resolution crystal structure of SHP in complex with a peptide derived from EID1. Unexpectedly, the EID1 peptide is bound to a interface on SHP and mimics the canonical helix H1 of nuclear receptor LBDs. Mutations in this interface affected SHP–EID1 interactions and decreased SHP repressor activity. Furthermore, the SHP–EID1 interface is highly conserved across species, suggesting that their interaction is biologically relevant. Together, our results reveal an interface that regulates the SHP–EID1 interaction and point to a potential molecular mechanism by which SHP recruits corepressors to exert its inhibitory function.

Results

EID1 Peptide Facilitates SHP Crystallization and Diffraction. SHP has been a difficult protein for structural studies due to its inherent insolubility when purified. Although initial strategies to express and purify SHP (including insect cell expression, mutations, coexpression with SHP cofactors, and GST fusion) were unsuccessful, ultimately we succeeded using a maltose binding protein (MBP) fusion strategy, which we and others have used extensively to improve protein solubility and crystallization (37, 38). Because the N-terminal region of SHP is not conserved across species (Fig. S1), we generated a series of mouse MBP-SHP proteins that contain SHP N-terminal truncations (named X1–X9 in Fig. L4 and S1). Interestingly, when SHP putative helices H1 and H2 were removed, MBP-SHP fusion proteins (X6–X9) became highly soluble, with a yield of 10–15 mg per liter of culture, which made crystallization possible (HisMBP-SHPLBD = X6 as an example in Fig. S2A).

One of the MBP-SHP constructs (X9, Fig. L4 and S1) yielded needle-like crystals in initial crystallization trials but not its longer counterparts (X6–X8). Extensive optimization failed to improve their quality. In an effort to overcome this issue, we replaced several surface residues of SHP with the corresponding residues of DAX-1, a closely related orphan receptor for which the crystal structure has been determined (39). This is the same general strategy that we used previously to crystallize the nematode nuclear receptor DAF-12 (8). Based on the sequence alignment of SHP and DAX-1 (PDB ID code 3FSC; sequence identity to SHP = 36%) (39), we mutated, either singly or in combination, a number of residues with flexible side chains that were predicted to be solvent-accessible to the corresponding DAX-1 residues that have less flexible side chains (Fig. L4). We then tested these mutant SHP proteins in crystallization and functional assays (Figs. S2B and S3). One mutant protein with amino acid changes at positions B, C, and E (Fig. L4), named MBP-SHPLBD(BCX), yielded bigger crystals that diffracted to 6.7 Å (Fig. S2B). These mutations did not affect the ability of SHP to repress LRH-1 activation or EID1 (Fig. S3). Repeated attempts to improve the diffraction limits of these crystals failed.

Inclusion of short peptides containing coactivator LXXLL motifs or corepressor LXXXLXXX(L/I) motifs has facilitated the crystallization and diffraction of a number of nuclear receptor LBDs (4, 8, 18). We thus examined the interaction of HisMBP-SHPLBD (X6, Fig. S2A) with a number of LXXLL- or LXXXLXXX(L/I)-containing peptides (refer to sequences in Table S1), but none of them interacted with SHP (Fig. 1B). It was reported previously that EID1, a conserved transcriptional repressor that inhibits p300/CBP coactivators, was able to interact with SHP via its middle domain (amino acids 54–120) (24). Accordingly, a number of biotinylated peptides that cover the mouse EID1 middle region were designed and tested in SHP-binding assays (Fig. 1 C and D). One EID1 peptide encompassing the sequence RVSAALEANKVFL bound to SHP (IC50 = 5–10 μM in a homologous competition assay), whereas the same peptide in which the NKVFL sequence was deleted...
(EID1-ΔNKVF) did not (Fig. 1C). We also tested the HisMBP fused full length SHP protein (SHPFL). The binding results in Fig. 1C indicated that the deleted putative SHP helices H1 and H2 did not affect EID1 binding by comparing SHP-LBD and SHPFL. Identification of the EID1 peptide is key to SHP crystallization because, when the peptide was included during crystallization, diffraction of MBP-SHPΔEID1 (refer to above and Fig. L4) crystals was improved to 2.8 Å, which allowed us to determine its structure (Fig. 1E).

**Crystal Structure of the SHP–EID1 Complex.** The SHP–EID1 structure was solved by molecular replacement using MBP as a model (see statistics in Table 1), which revealed that each noncrystallographic asymmetric unit contained one complex (Fig. 2A). The overall architecture of SHP is similar to that of DAX-1 (Fig. 2B). SHP helix H12 occupies its own AF-2 site by mimicking coactivator LXXLL motifs (Fig. 2C). A clear kink is observed between helices H10 and H11, which results in the collapse of helix H11 into the space that corresponds to the ligand-binding pocket of ligand-regulated receptors (compare Fig. 2B and C), leaving no room for ligand binding. Thus, the SHP structure is in an auto-repressed and ligand-free conformation, resembling those of several other orphan nuclear receptors, including DAX-1, COUP-TFII, TR4, and PNR (Fig. 2B and Fig. S4) (38-41).

The most unexpected feature of the SHP–EID1 structure is the binding mode of EID1. Instead of binding to the canonical AF-2 site of SHP, EID1 is bound to SHP by mimicking helix H1 of the nuclear receptor LBD, which is illustrated by superposition of SHP to the ligand-bound RXR LBD (Fig. 2C). We termed the SHP EID1-binding pocket as the helix H1 pocket to distinguish it from the classic AF-2/H12 pocket. The SHP/EID1-binding site consists of residues from SHP helices H3, H5, H8, and H9 that form a hydrophobic patch to adopt the EID1 helix (Fig. 2D and Fig. S5A). The SHP residues that interact with EID1 include six hydrophobic residues (F72, L76, A170, Y171, F178, and L194) and two hydrophilic residues (Q105 and K173), which form hydrogen bonds with EID1. The EID1 residues that interact with SHP include four hydrophobic residues (V94, L98, A101, and F105) and one hydrophilic residue (N102), which hydrogen-bond with SHP (Fig. 2D and Fig. S5A). The SHP and EID1 residues that mediate the SHP–EID1 interaction are conserved across species (Figs. S1 and S5B), suggesting that their interaction is biologically relevant. Interestingly, although the SHP residues are also conserved in DAX-1 (Fig. 1A), and although SHP shares a similar 3D structure with DAX-1, EID1 did not bind to DAX-1 (Fig. S6A), indicating that the SHP–EID1 interface is receptor-specific.

**Analysis of the SHP–EID1 Interaction.** To determine the role of SHP interface residues in EID1 binding, we mutated individual EID1-contacting residues in SHP (Fig. 3A) and measured the effects on SHP–EID1 interactions in AlphaScreen binding assays. As shown in Fig. S6B, most of these mutated proteins (except for SHP F178S) retained their ability to interact with LRH-1, indicating that they retained their functional folds. In contrast, all mutations severely affected SHP binding to the EID1 peptide, consistent with a critical role for these residues in EID1 recognition (Fig. 3B). The results were further confirmed in mammalian two-hybrid assays (Fig. 3 C and D). Mutations of EID1-contacting residues in the full-length SHP (Fig. 3C) or SHP-contacting residues in EID1 (Fig. 3D) abolished SHP–EID1 interactions.

**Functional Analysis of the SHP–EID1 Interface.** Next, we investigated the importance of the integrity of the SHP–EID1 interface in SHP repression. SHP inhibits LRH-1 and HNF4α transcriptional activity (13–15). Hence we used Gal4-LRH-1 and Gal4-HNF4α to study repressor activity of SHP wild type (WT) and mutants. Mutations of F72, F178, and L76 decreased SHP function significantly on LRH-1 and HNF4α (Fig. 4 A and B). These mutations did not change SHP expression level or its ability to bind the receptor (Fig. 4 C and D), suggesting that the mutations

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**Table 1. Data collection and refinement statistics for MBP-SHP/EID1**

<table>
<thead>
<tr>
<th>Data collection</th>
<th></th>
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<tr>
<td>Space group</td>
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<td>Resolution, Å</td>
<td>30.28</td>
</tr>
<tr>
<td>Cell parameters a, b, c, Å</td>
<td>56.39, 105.15, 136.28</td>
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<tr>
<td>β, °</td>
<td>90</td>
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<tr>
<td>Total reflections</td>
<td>205,691</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>20,723</td>
</tr>
<tr>
<td>Rsym</td>
<td>0.176 (0.754)</td>
</tr>
<tr>
<td>Rfree, %</td>
<td>12.8 (2.2)</td>
</tr>
<tr>
<td>Completeness, %</td>
<td>100</td>
</tr>
<tr>
<td>Redundancy, %</td>
<td>9.9 (8.2)</td>
</tr>
</tbody>
</table>

Structure determination and refinement

| Resolution, Å | 30.28      |
| No. of reflections | 17,181    |
| No. of residues     | 605       |
| No. of solvent molecules | 49      |
| No. of non-H atoms  | 4,493     |
| Rwork, %            | 19.46     |
| Rfree, %            | 24.12     |
| rmsd bonds, Å       | 0.009     |
| rmsd angles, °      | 1.07      |
| Average B factor, Å² | 15.1     |

Values for the highest-resolution shell (2.9–2.8Å) are given in parentheses.
impaired SHP’s ability to recruit corepressors. Finally, we examined the conservation of the SHP–EID1 interface by including SHP sequences from additional species that are available in the protein database (32 species in total; refer to Table S2). As illustrated in the protein conservation map (Fig. 4F), the amino acids that form the EID1-binding pocket are highly conserved, particularly F72, Q105, A170, and F178.

Discussion

SHP is an atypical nuclear receptor in that it lacks a DBD but is able to interact directly with other nuclear receptors and to repress their transcriptional activity. These characteristics make SHP an intriguing target for structural studies (11). However, determining the structure of SHP has proven difficult due to solubility issues. In this study, we overcame these difficulties by using MBP fusion and domain-mapping strategies, which allowed us to obtain large amounts of soluble SHP protein for crystallization and biochemical analysis. Through a combination of N-terminal deletions, surface mutations, and inclusion of an EID1 peptide, we were able to crystallize the SHP–EID1 complex and solve its structure. The structure reveals an unexpected SHP–EID1 interface, where EID1 adopts a helical structure that mimics helix H1 in the canonical LBD fold. Mutations in the SHP EID1 interface, where EID1 adopts a helical structure that mimics helix H1 in the canonical nuclear receptor LBD fold. Instead, the EID1 peptide mimics the formation of helices. Second, repeated attempts to crystallize full-length SHP-containing putative H1 and H2 sequences have failed. Third, our cocystal structure of SHP and the EID1 peptide reveals that SHP lacks helix H1 of the canonical nuclear receptor LBD fold. Instead, the EID1 peptide mimics the formation of helices.

Implications for SHP Cofactor Recruitment

SHP functions predominantly as a transcriptional repressor as exemplified by its physiological role in interacting with LRH-1 and HNF4a to inhibit CYP7A1 expression and bile acid synthesis. A number of proteins have been isolated as SHP corepressors, including histone deacetylases (SIRT1, HDAC1, and HDAC3) (25, 28) and EID1, which inhibits p300/CPB HAT activity (24, 26, 27). However, the mechanism whereby these corepressors interact with SHP has been poorly understood. An important clue to unraveling this mechanism comes from our sequence analysis and crystallization experiments indicating that SHP does not have canonical H1 and H2 helices. First, the N-terminal amino acids corresponding to H1 and H2 are not conserved across species and contain many prolines that disfavor the formation of helices. Second, repeated attempts to crystallize full-length SHP-containing putative H1 and H2 sequences have failed. Third, our cocystal structure of SHP and the EID1 peptide reveals that SHP lacks helix H1 of the canonical nuclear receptor LBD fold. Instead, the EID1 peptide mimics the formation of helices. EID1 binds to SHP in an unexpected manner that mimics the formation of helices. Although the mechanism of SHP repressor activity based on our studies.

It is of interest to note that not all of the mutations in SHP that affected EID1’s interaction (Fig. 3 C and D) resulted in loss of repression activity (24, 26, 27). However, the mechanism whereby these corepressors interact with SHP has been poorly understood. An important clue to unraveling this mechanism comes from our sequence analysis and crystallization experiments indicating that SHP does not have canonical H1 and H2 helices. First, the N-terminal amino acids corresponding to H1 and H2 are not conserved across species and contain many prolines that disfavor the formation of helices. Second, repeated attempts to crystallize full-length SHP-containing putative H1 and H2 sequences have failed. Third, our cocystal structure of SHP and the EID1 peptide reveals that SHP lacks helix H1 of the canonical nuclear receptor LBD fold. Instead, the EID1 peptide mimics the formation of helices.
of repression (Fig. 4A and B). A likely explanation for this difference is that SHP recruits other corepressors through the helix H12 site that act independently of EID1. Indeed, previous studies have shown SHP helix H12 is crucial for its repressor activity (24), and EID1 is known to form a multiple-component complex with other corepressors to regulate SHP’s inhibitory activity (20, 22, 23). For this reason, mutation of the SHP-EID1 interface residues F72, L76, and F178 might be expected to compromise both EID1 binding and repression activity, whereas mutations of other SHP–EID1 interface residues may still retain SHP repressor activity because they permit the assembly of other non-EID1–dependent corepressors through the H12 helix. Another possible explanation is that SHP may recruit a different set of corepressors through the same interface that requires F72, L76, and F178 as key residues mediating these interactions. Validation of these potential interactions will require future experimentation.

The molecular basis for SHP–EID1 interactions is very different from that for interactions between traditional ligand-regulated receptors and corepressors such as NCoR and SMRT. In the case of ligand-regulated receptors, antagonist binding displaces the C-terminal helix H12 from the canonical LBD fold, thus opening the AF-2 pocket for the binding of the LXXLXXX (L/I) motifs present in NCoR and SMRT (10). In the case of SHP, its AF-2 site is occupied by its own helix H12. Instead, SHP uses a helix H1 pocket to accommodate EID1, which lacks the classical corepressor LXXLXXX (L/I) motif. Several other orphan nuclear receptors such as DAX-1, TR4, TLX, and PNR function primarily as transcription repressors to regulate diverse physiological programs (2, 42, 43). These orphan nuclear receptors directly recruit receptor-specific corepressors instead of common corepressors such as SMRT and NCoR (17, 24, 42–45). Although the mechanisms of cofactor recruitment by these orphan nuclear receptors are unclear, the known corepressors for these receptors do not contain LXXL or LXXLXXX (L/I) motifs. Based on these observations, we speculate that the H1 helix pocket may be the alternative to the AF-2 site for the recruitment of corepressors by these specialized orphan receptors. Consistent with this notion, repressor orphan nuclear receptors DAX-1, TR4, and PNR also lack an H1 helix (Fig. 2B and Fig. S4B and C) (38, 39, 41), thus providing an interaction surface for recruitment of their corresponding corepressors. It is worth noting that for ligand-regulated receptors, the isolated helix H1 pocket can be assembled in trans with the rest of the LBD in a ligand-independent manner, and further, ligand binding is enhanced by the inclusion of helix H1 in the canonical LBD (46).

It is noteworthy that our SHP structure is a ligand-free structure with helix H10 collapsed into the ligand-binding pocket, much like the apo-RXR structure (47, 48). Interestingly, synthetic retinoid-like compounds such as 3-Ci-AHPC have been shown to bind and regulate SHP activity (34–36). We speculate that binding of these pharmaphores might rearrange the kinked helix H10, thus allowing formation of the AF-2 cofactor-binding pocket. Consistent with this hypothesis, 3-Ci-AHPC can promote SHP interaction with LXXL-containing peptides (Fig. S7A). In contrast, EID1 binding to SHP is independent of the presence of 3-Ci-AHPC (Fig. S7B). This suggests that SHP has two cofactor-binding sites, including one that is ligand-dependent (and thus potentially druggable) via the C-terminal AF-2 site and the other that is ligand-independent via the EID1-binding site near the helix H1 pocket. Thus, our SHP–EID1 structure complex provides an explanation for SHP cofactor recruitment and repressor function, and it reveals a protein interface that regulates nuclear receptor functions.

Materials and Methods

Plasmids and Reagents. The mutants used for crystallization, AlphaScreen assays, or cotransfection assays were created by site-directed mutagenesis using the QuikChange method (Stratagene) and verified by sequencing. Protein Preparation and Crystallization. For biochemical assays, mouse SHP (amino acids 46–260) was cloned into an engineered pETDuet-1 (Novagen) plasmid, in which HisMBP was introduced in front of its original multiple cloning sites. HisMBP-SHPFL (amino acids 1–260; the last 10 cysteines were mutared to serines for solubility) and HisMBP-DAX1-LBD (NP_031456, amino acids 205–472) were cloned in the same way. Biotinylated MBP-LRH1 (NP_995582, amino acids 299–541) was constructed as described elsewhere (49). For crystallization trials, SHP (amino acids 55–260) was cloned into the MBP vector described previously (37). The proteins were expressed in BL21 (DE3) cells, first purified by amylose-resin chromatography (Biolabs) and then followed by size-exclusion chromatography.

The MBP-SHP crystals were grown at 20 °C in sitting drops containing 1.0 μL of the protein solution (10 mg/mL) and 1.0 μL of the well solution containing 0.1 M TrisCl, pH 8.5, 25% (v/vol) PEG1000. The MBP-SHP/EID1 crystals were grown at 20 °C in sitting drops containing 1.0 μL of the protein solution (10 mg/mL) and 1.0 μL of the well solution containing 0.1 M 2-(cyclohexylamino)ethanesulfonic acid, pH 9.5, 30% (v/vol) PEG3000. The molecular ratio of MBP–SHP to the EID1 peptide (YSGAMHRVSAA–LEEANKVFLRTARAGDALG) was 1:1.5. In general, crystals appeared within 2 d and grew to the final size in about 1 wk, at which time they were flash-frozen and stored in liquid nitrogen.

Data Collection, Structure Determination, Refinement, Superposition, and Conservation Heat Map. The diffraction data were collected with a MAR225 CCD detector at the 21-ID beamline at the Advanced Photon Source at Argonne National Laboratory (Argonne, IL). The observed reflections were reduced, merged, and scaled with DENZO and SCALEPACK in the HKL2000 package. The structure was determined with the PHASER program by molecular replacement using the crystal structures of MBP and DAX-1 as the models. Manual model building was carried out with the programs O (50) and COOT (51), and the structure was refined with crystallography NMR software (52) and the CCP4 program refmac5 (53). The conservation heat map was compiled from 32 SHP sequences using the program Consurf (http://consurf.tau.ac.il) (54). The data collection and structure determination statistics are summarized in Table 1.

AlphaScreen Binding Assays. The binding of the cofactor peptides to SHP was determined by AlphaScreen luminescence proximity assays as described (8, 18, 41). Reaction mixtures consisted of 50 nM HisMBP fusion proteins, 200 nM biotinylated peptides/proteins, 10 μg/mL nickel chela-te-coated acceptor beads (PerkinElmer Life Sciences), and 10 μg/mL streptavidin-coated donor beads (PerkinElmer Life Sciences) in a buffer containing 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS), pH 7.4, 50 mM NaF, 50 mM 3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonate (CHAPS), and 0.1 mg/mL BSA. The peptides used in our studies are listed in Table S1.

Cell Reporter Assay and Statistics Analysis. AD293 cells were cultured and cotransfected in 24-well plates as reported (41). Mouse SHPFL (amino acids 1–260) WT and mutants were cloned into the p3xFLAG-CMV expression vector (Sigma, E4401). A GSAGSA linker was added in front of the SHP gene. VP16-SHPFL was constructed by fusing mouse SHP (amino acids 1–260) to the VP16 vector. Gal4-EID1 was constructed by fusing mouse EID1 middle domain (amino acids 54–120) to the Gal4 vector. Gal4-LRH-1 and HNF4a were constructed as described elsewhere (15). Statistical analysis was performed using Excel (38). Comparisons were performed using Student’s independent-sample t test (two-tailed distribution and two-sample equal variance). The statistical significance level was set at P < 0.05 or P = 0.01.

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