Supporting Information

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SI Materials and Methods

Protein Preparation. Proteins fused to maltose binding protein (MBP) or MBP with a histidine tag in front of its N terminus (HisMBP) were expressed in *Escherichia coli* BL21 (DE3) following the same general method as described previously (1). Briefly, proteins were first purified by maltose affinity chromatography, followed by gel filtration chromatography (Superdex 200) in 10 mM Tris, pH 8.0, 100 mM NaCl, 5% (vol/vol) glycerol, 1 mM EDTA, and 1 mM DTT. We generated a total of 17 sets of MBP-small heterodimer partner (SHP) (E60A, E85D, L126T/E127T/E128R, E207R, K228R, E251D, E254L, E85D/L126T/E127T/E128R/E207R, E85D/L126T/E127T/E128R/K228R, L126T/E127T/E128R/E207R/K228R, E85D/E207R/K228R, E251D/E254L, E60A/E251D/E254L, E251D/E254L/E85D/L126T/E127T/E128R/E207R, E251D/E254L/E85D/L126T/E127T/E128R/K228R, E251D/E254L/L126T/E127T/E128R/E207R/K228R, and E251D/E254L/E85D/L126T/E127T/E128R/E207R/K228R) homologous mutants. Mutated residues were predicted to be solvent-exposed and have flexible side changes based on the position of the homologous amino acids in the DAX-1 structure (2). Only MBP-SHPE85D/ L126T/E127T/E128R/K228R yielded improved crystals. To obtain the soluble full-length MBP-SHP protein, the last 10 cysteines in mouse SHP were mutated to serines.

Protein Crystallization. Before crystal screen, MBP proteins were supplemented with 1 mM maltose to stabilize MBP. MBP-SHP proteins alone or mixed with the E1A-like inhibitor of differentiation (EID1) peptide (YSGAMHRVSAALEEANKVFLRTARAGDALDG) at a molar ratio of 1:1.5 were applied to Hampton and Qiagen crystal screens. The crystals appeared within 2 d and grew to the final size in about 1 wk. Then they were mounted to the appropriate loops with 20% ethylene glycol and flash-frozen.

AlphaScreen Assays. Interactions between SHP and EID1 (or LRH-1) were assessed by luminescence-proximity AlphaScreen technology as described previously (3). Reaction mixtures consisted of 50 nM HisMBP fusion proteins, 200 nM biotinylated peptides/proteins, 10 μg/mL nickel chelate-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.

Cell Reporter Assays. Plates (24-well) containing AD293 cells were used to measure SHP repressor activity. Each well contained 100 ng of pG5-luc (Promega), 1 ng of Renilla-luc (transfection control), 50 ng of Gal4-LRH-1 or HNF4α, and Flag-SHP. Ten nanograms of Flag-SHP was used to repress Gal4-LRH-1 and 50 ng of Flag-SHP was used to repress Gal4-HNF4α. Two days after transfection, the cells were lysed following the protocol in the dual-luciferase reporter assay system (Promega) kit. Firefly luciferase (from pG5-luc) was measured first and then Renilla luciferase. Relative light unit (RLU) was calculated by dividing Firefly luciferase by Renilla luciferase.

1. Pioszak AA, Xu HE (2008) Molecular recognition of parathyroid hormone by its G protein-coupled receptor. *Proc Natl Acad Sci USA* 105(13):5034–5039.
2. Sablin EP, et al. (2008) The structure of corepressor Dax-1 bound to its target nuclear receptor LRH-1. *Proc Natl Acad Sci USA* 105(47):18390–18395.
3. Zhi X, et al. (2012) Structural conservation of ligand binding reveals a bile acid-like signaling pathway in nematodes. *J Biol Chem* 287(7):4894–4903.

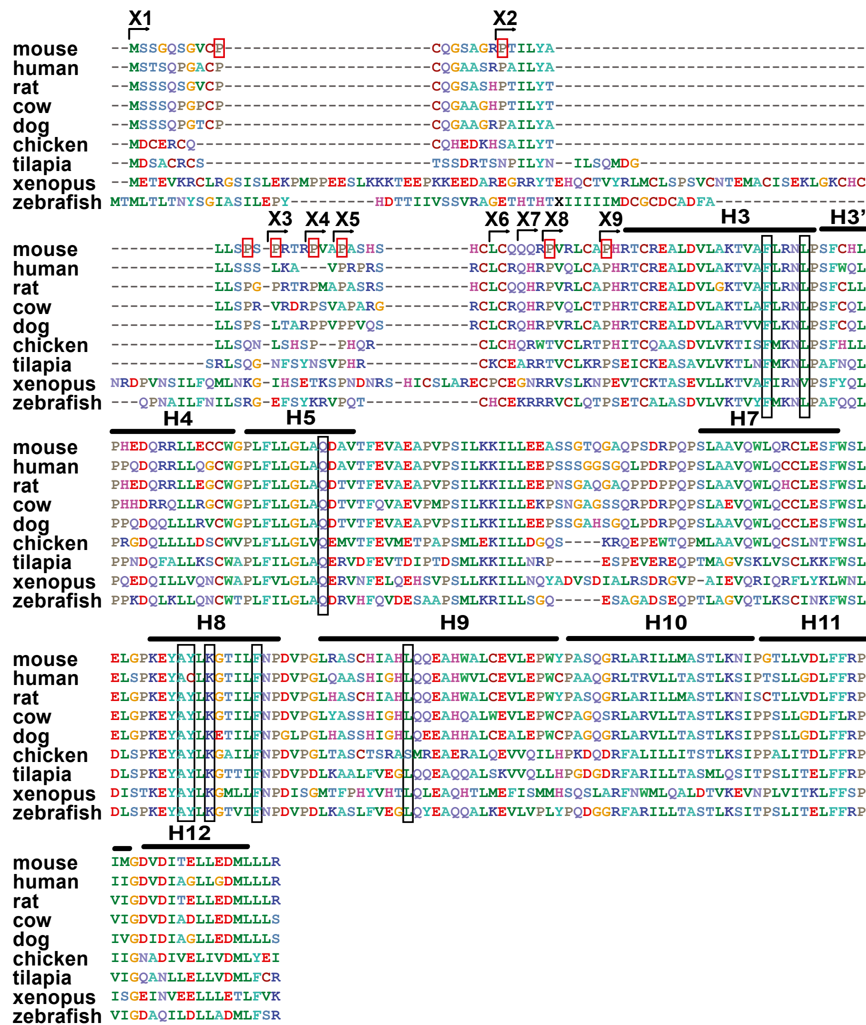

Fig. S1. The full-length sequence alignment of SHP from mouse (NP_035980), human (NP_068804), rat (NP_476474), cow (XP_002685805), dog (XP_854945), chicken (NP_001026064), tilapia (XP_003452125), *Xenopus* (XP_002936259), and zebrafish (NP_001243120). A series of mouse MBP-SHP N-terminal truncation constructs were made (designated by arrowheads and named the letter "X" followed by numbers). Constructs X6–X9 were expressed to be soluble and then tested in crystallization. MBP-SHPX9 yielded the initial needle-like crystals. The positions of several prolines N-terminal to helix H3 in the mouse SHP protein sequence that are conserved across species are highlighted by red boxes. Helices H3-H12 are labeled by H and numbers. Traditional helices H1–H2 are not present in SHP. The amino acids involved in SHP/EID1 binding are conserved and highlighted by black boxes.

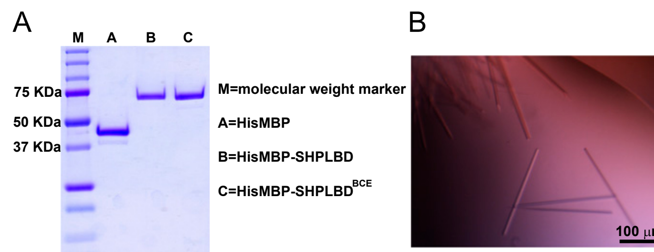

Fig. S2. Protein expression and crystallization of SHP. (*A*) Coomassie Blue-stained SDS/PAGE gel with ~1 µg of soluble HisMBP protein per lane. HisMBP-SHP LBD is mouse SHP (amino acids 46–260) fused to HisMBP. HisMBP-SHPLBD^{BCE} is mouse SHP (amino acids 46–260), containing the combination of mutations indicated in Fig. 1*A*, fused to HisMBP. (*B*) Representative picture of MBP-SHP^{BCE} crystals that diffracted up to 6–7 Å.

Fig. S3. Functional analysis of SHP mutants. (*A*) Gal4-LRH-1 was used in cell reporter assays to evaluate the effect of SHP mutations (highlighted in Fig. 1*A*) on SHP repressive activity. SHP^{BCE} that produced crystals for structure determination showed comparable activity to SHP wild type. RLU, relative light units, were normalized with *Renilla* luciferase activity as the transfection control. The numbers indicate repression fold and were calculated by comparing RLU with SHP to RLU without SHP. Error bars = SD ($n = 3$). (*B*) Wild-type SHP and SHP^{BCE} proteins bound equally well to the EID1 peptide in AlphaScreen assays, using the purified proteins shown in Fig. S2*A*. Error bars = SD ($n = 3$).

Fig. S4. Structural comparison between SHP and other orphan nuclear receptors. (*A*) Superposition of SHP (green) to COUP-TF2 (white, PDB ID code 3CJW). (*B*) Superposition of SHP (green) to TR4 (light pink, PDB ID code 3P0U). (*C*) Superposition of SHP (green) to PNR (light yellow, PBD ID code 4LOG).

Fig. S5. A conserved EID1 sequence interacts with SHP. (*A*) Schematic presentation of the interactions between the EID1 peptide and SHP. EID1 residues are labeled in yellow and SHP residues in green. Hydrophobic interactions are illustrated by solid lines and H-bonds by dashed lines. Numbers in parentheses indicate the interaction distances in angstroms. When two numbers are listed in parentheses, the first number shows the interaction distance to the upper interacting residue and the second number the distance to the lower interacting residue. (*B*) The sequence alignment of the EID1 motif bound to SHP. Numbers refer to the amino acid positions in EID1 proteins from mouse (NP_079889), human (NP_055150), rat (NP_001102675), cow (NP_001091847), dog (XP_850599), sheep (XP_004010681), armadillo (XP_004448130), walrus (XP_004396587), and whale (XP_004281351). The residues interacting with SHP are designated by arrows.

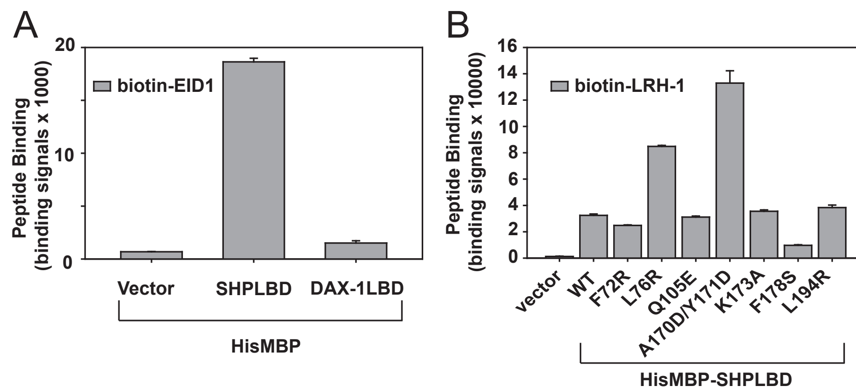

Fig. S6. The SHP–EID1 interface is receptor-specific and different from the SHP–LRH-1 interface. (*A*) EID1 does not bind to DAX-1. Error bars = SD ($n = 3$). (*B*) Mutations of SHP–EID1 interface residues do not abolish the SHP–LRH-1 interaction. The same proteins that were used to generate the data in Fig. 3*B* were tested in AlphaScreen binding assays against biotinylated MBP-LRH-1. Error bars = SD ($n = 3$).

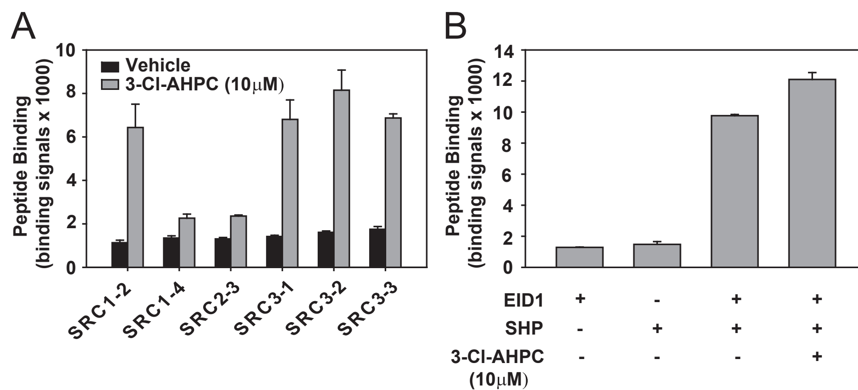

Fig. S7. 3-Cl-AHPC promotes SHP interaction with specific LXXLL-containing peptides. (*A*) HisMBP-SHP LBD binds to biotinylated SRC1-2, SRC3-1, SRC3-2, and SRC3-3 LXXLL-containing peptides in the presence of 3-Cl-AHPC (10 μM) in in vitro ligand-binding assays. Error bars = SD ($n = 3$). (*B*) 3-Cl-AHPC does not change SHP interaction with the biotinylated EID1 peptide. HisMBP-SHP LBD and the biotinylated EID1 peptide were used in assays. Error bars = SD ($n = 3$).

Table S1. Sequence of peptides used in AlphaScreen assays

Peptide	Sequence
EID1	YSGAMHRVSAALEEANKVFLRT
EID1-ΔNKVFL	YSGAMHRVSAALEEA
SRC1-2	SPSSHSSLTERHKILHRLLQEGSP
SRC1-4	QKPTSGPQTPQAQQKSLLQQLLTE
PGC1α-1	QEAEEPSLLKKLLLAPANTQ
TRAP-1	GHGEDFSKVSQNPILTSLLQITGN
CBP-1	SGNLVPDAASKHKQLSELLRGGSG
NcoR-2	GHSFADPASNLGLEDIIRKALMGSF
SHP-1	PCQGSASHPTILYTLLSPGP
SHP-2	VAEAPVPSILKKILLEEPNS
SMRT-2	ASTNMGLEAIIRKALMGKYDQ
SRC2-3	QEPVSPKKKENALLRYLLDKDDTKD
SRC3-1	AENQRGPLESKGHKKLLQLLTSS
SRC3-2	TSNMHGSLLQEKHRILHKLLQNG
SRC3-3	KENNALLRYLLDRDD

Table S2. SHP species and protein ID

Species	Protein ID
Human	NP_068804
Mouse	NP_035980
Rat	NP_476474
Cow	XP_002685805
Dog	XP_854945
Chimpanzee	XP_001146990
Monkey	XP_003921311
Sheep	XP_004005545
Cat	XP_003989795
Chicken	NP_001026064
Hedgehog	XP_004705389
Jerboa	XP_004671279
Degu	XP_004642914
Shrew	XP_004603770
Pika	XP_004592265
Armadillo	XP_004478819
Walrus	XP_004394749
Manatee	XP_004377304
Dolphin	XP_004318439
Whale	XP_004266673
Gorilla	XP_004025301
Baboon	XP_003891451
Galago	XP_003799855
Gibbon	XP_003271715
Marmoset	XP_002750522
Finch	XP_002191963
Mole	XP_004679455
Rhino	XP_004425848
Dasyuridae	XP_003765030
Tilapia	XP_003452125
Zebrafish	NP_001243120
Xenopus	XP_002936259