

SUPPLEMENTARY FIGURE LEGENDS

FIGURE S1. Specificity of oxPTP mAb for oxidized highly conserved PTP peptide surrounding the catalytic site cysteine. *A-B*, The peptides VHCSAG and its oxidized form ox-VHCSAG were prepared as described in Experimental procedures, and dissolved in PBS. Masses of VHCSAG (*A*) and ox-VHCSAG (*B*) were determined by mass spectrometry. *C*, mAb oxPTP in PBS (Con.) or in PBS with different concentrations of VHCSAG or ox-VHCSAG peptides was used for immunoblotting detection of oxidized phosphatases in size-fractionated postnuclear supernatants from Triton X-100-solubilized RBL cells activated for 15 min with 0.2 mM pervanadate. The blots were analyzed by densitometry and the data obtained were normalized to a control without peptide (Fold). Numbers on the right indicate positions of molecular weight markers in kDa. Two immunoblotting experiments were performed with similar results.

FIGURE S2. Oxidation of immunoprecipitated PTPs. BMMCs were activated by Pv (0.2 mM, 15 min), and SHP-1, SHP-2, HePTP and PTP-MEG2 (MEG2) were immunoprecipitated with the corresponding antibodies. The immunoprecipitated PTPs together with total cell lysates (Total) were size-fractionated by SDS-PAGE and analyzed by immunoblotting with oxPTP mAb (left) or PTP-specific antibodies (right). Position of individual phosphatases is indicated on the right. It should be noted that in fractionated total cell lysate, oxPTP mAb reacts weakly with SHP-2 and strongly with SHP-1, which has lower molecular weight.

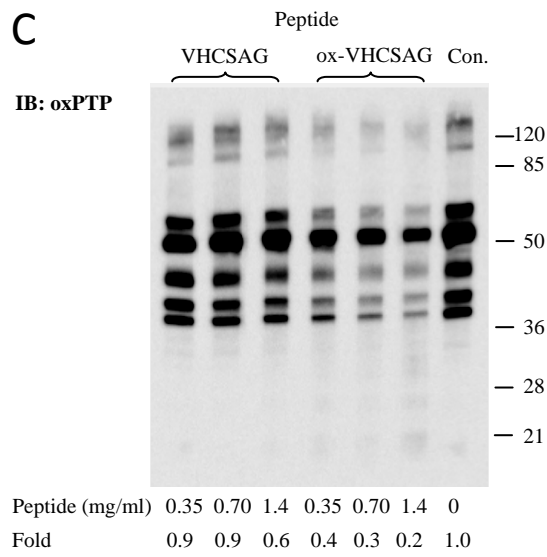
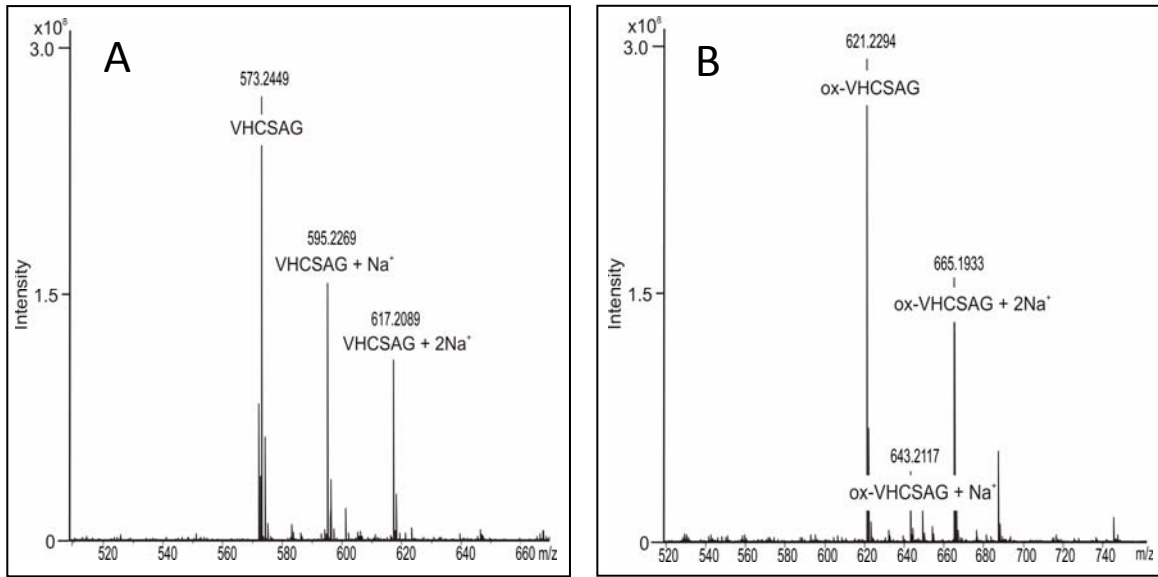


Figure S1
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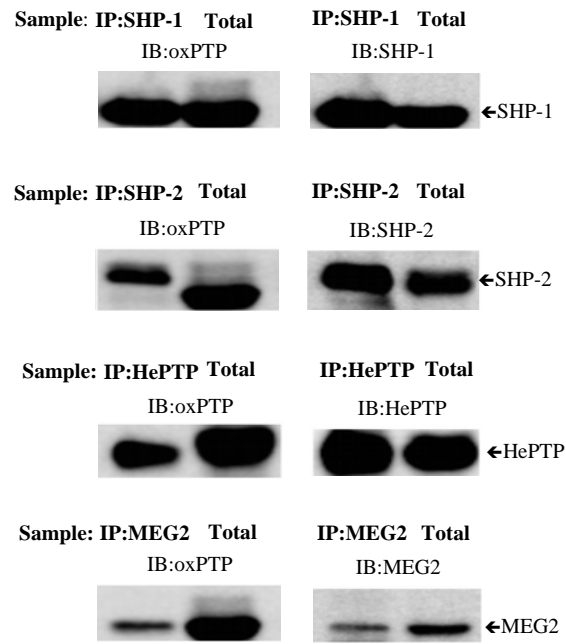


Figure S2
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TABLE S1
Mass spectrometry identification of SHP-1 in oxPTP mAb immunoprecipitate

Measured mass [M+H] ⁺	Computed mass [M+H] ⁺	Peptide sequence	Peptide position
897.4352	897.4344	R.QPYYATR.V	211 - 217
902.4982	902.4974	R.GVPGSFLAR.P	22 - 30
991.4257	991.4247	R.EHDTAEYK.L	384 - 391
1069.5526	1069.5516	R.LEGQRPENK.S	263 - 271
1081.5512	1081.5512	R.VDDQVTHIR.I	45 - 53
1097.5616	1097.5618	K.NILPFDHSR.V	278 - 286
1103.576	1103.5764	K.GEPWTFLLVR.E	128 - 136
1121.5462	1121.5465	K.NQGDFSLSVR.V	35 - 44
1240.5762	1240.5758	R.SGMVQTEAQYK.F	496 - 506
1257.6349	1257.6353	R.VYGLYSVTNSR.E	373 - 383
1437.6204	1437.6194	K.YPLNCSPTSER.W	98 - 109
1469.6829	1469.6827	K.AGFWEFESLQK.Q	242 - 253
1490.6969	1490.6976	K.CVPYWPEVGTQR.V	361 - 372
1511.7603	1511.762	K.TGIEEASGAFVYLR.Q	197 - 210
1539.8256	1539.8257	R.TLQISPLDNGDLVR.E	394 - 407
1642.8991	1642.897	K.FIYVAIAQFIETTK.K	507 - 520
1704.7631	1704.7631	R.IQNSGDFYDLYGGEK.F	54 - 68
1768.8305	1768.8268	R.DSNIPGSDYINANYVK.N	293 - 308
1779.8456	1779.8428	K.GQSEYGNITYPPAVR.S	531 - 546
1980.9829	1980.9806	R.WYHGHISGGQAESLLQAK.G	110 - 127
2072.0445	2072.0426	R.ESLSQPGDFVLSVLNDQPK.A	137 - 155
2244.0541	2244.0587	R.YTVGGSETFDSLTDLVEHFK.K	176 - 195
2372.1611	2372.1536	R.YTVGGSETFDSLTDLVEHFKK.T	176 - 196
2515.2648	2515.2595	K.FATLTELVEYYTQQQILQDR.D	69 - 89

1 MVRWFHRDLS GPDAETLLKG RGVPGSFLAR PSRKNQGDFS LSVRVDDQVT
51 HIRIQNSGDF YDLYGGEKFA TLTELVEYYT QQQILQDRD GTIIHLKYPL
101 NCSPTSERW YHGHISGGQA ESLLQAKGEP WTFLLVRESLS QPGDFVLSVL
151 NDQPKAGPGS PLRVTHIKVM CEGGRYTVGG SETFDSLTDL VEHFKKTGIE
201 EASGAFVYLR QPYYATRVNA ADIENRVLEL NKKQSESDTA KAGFWEEFES
251 LQKQEVKLNH QRLEGQRPEN KSKNRYKNIL PFDHSRVILQ GRDSNIPGSD
301 YINANYVKNQ LLGPDENSKT YIASQGCLDA TVNDFWQMAW QENTRVIVMT
351 TREVEKGRNK CVPYWPEVGT QRVYGLYSVT NSREHDTAEY KLRTLQISPL
401 DNGDLVREIW HYQYLSWPDH GVPSEPGGVL SFLDQINQRQ ESLPHAGPII
451 VHCSAGIGRT GTIIVIDMLM ESISTKGLDC DIDIQKTIQM VRAQRSGMVQ
501 TEAQYKFIYV AIAQFIETTK KKLEIIQSOK GQSEYGNIT YPPAVRSAHA
551 KASRTSSKHK EEVYENVHSK SKKEEKVKKQ RSADKEKNKG SLKRK

The cells were activated by pervanadate (0.2 mM, 15 min) and oxidized PTPs were immunoprecipitated with oxPTP mAb and analyzed by mass spectrometry as described in Experimental procedures. Measured and computed masses, peptide sequences and peptide positions are shown. Amino acids of the identified peptides in the mouse SHP-1 (UniProtKB/Swiss-Prot P29351) are indicated in bold and underlined (bottom part of the table).

TABLE S2

Mass spectrometry identification of SHP-2 in oxPTP mAb immunoprecipitate

Measured mass [M+H] ⁺	Computed mass [M+H] ⁺	Peptide sequence	Peptide position position
1440.7016	1439.6943	R.QLTIQHQQECSR.S	214 - 224
1471.7964	1470.7892	R.GEVDILGIVCQLR.L	293 - 305
1645.8041	1644.7968	R.IQDMKEHPEYTVR.Q	201 - 213
2050.0145	2049.0072	K.IPSNPFVNPEDLDIPGHASK.D	83 - 101
2282.0193	2281.012	K.CVHYWPTEEEAYGPFQIR.I	183 - 200
2372.2179	2371.2106	K.HILFSAWPDHQTPESAGPLLR.L	228 - 248

1 MTQPPPTKAP AKKHVRLQER RGSSVALMLD VQSLGTVEPI CSVNTPREVT
51 LHFLRTAGHP LTRWTLQHQP PSPKQLEEEF LK**IPSNFVNP EDLDIPGHAS**
101 **K**DRYKTIILPN PQSRVCLGRA QSQEDSDYIN ANYIRGYDGK EK**VYIATQGP**
151 MPNTVADFWE MVWQEDVSLI VMLTQLREGK EK**CVHYWPTE EEAYGPFQIR**
201 **IQDMKEHPEY TVRQLTIQHQ QECSR**VKHIL **FSAWPDHQTP ESAGPLLR**LV
251 AEVETPETAA NSGPIVVHCS AGIGRTGCFI ATRIGCQQLK AR**GEVDILGI**
301 **VCQLRL**DRGG MIQTAEQYQF LHHTLALYAA QLPPEPNP

The cells were activated and analyzed as in Table S1. Amino acids of the identified peptides in the mouse SHP-2 (UniProtKB/Swiss-Prot Q8BUM3) are indicated in bold and underlined (bottom part of the table).