

## Supporting Information

# High-Throughput Screening of Catalytically Inactive Mutants of Protein Tyrosine Phosphatases (PTPs) in a Phosphopeptide Microarray

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### 1. Materials and Methods

**1.1 Materials.** All chemicals were purchased at the highest grade and used without further purification, unless otherwise noted. Fmoc-amino acids, HOBt, HBTU, TIS, TFA were from GL Biochem (China). Piperidine was from Merck (USA). HPLC profiles and ESI mass spectra were acquired in the positive or negative mode by using a Shimadzu IT-TOF. Analytical and semi-preparative RP-HPLC separations were performed on Phenomenex C<sub>18</sub> analytical (150 x 3.0 mm) and semi-preparative (250 x 21.2 mm) columns, respectively, using a Shimadzu Prominence HPLC system equipped with a Shimadzu SPD-20A detector. Eluents A (0.1 % TFA/acetonitrile) and B (0.1 % TFA/water) were used as the mobile phases. PTP proteins were expressed and purified in house. Plain glass slides were purchased from Sigma Aldrich (USA), and modified to generate the corresponding avidin-coated surface as previously described.<sup>1</sup> Pro-Q<sup>TM</sup> Diamond dye was bought from Invitrogen (USA).

**1.2 Microarray preparation.** All peptide stocks were prepared in an 1:1 DMSO/PBS spotting solution (to 1 mM final concentration), and were distributed in 384-well plates. All peptides were shown to be completely soluble in this spotting solution. Previously, we have shown that 500  $\mu$ M spotting concentration of peptides was sufficient to completely saturate available biotin-binding surface.<sup>1</sup> Slides were spotted on an ESI SMA arrayer (Ontario, Canada) with the printhead installed with 8 Stealth SMP8B Microspotting pins (Telechem, U.S.A.). Spots generated were of approximately 350  $\mu$ m diameter and were printed with a spot-spot spacing of 450  $\mu$ m. The pins were rinsed in between samples using two cycles of wash (for 10 s) and sonication (for 10 s) in reservoirs containing 70 % ethanol followed by drying under reduced pressure (for 10 s). The slides were allowed to stand for 1 h on the printer platform and stored at 4 °C until use. Before incubation with the labeled protein, the slides were rinsed with TBS (pH 7.4) for 10 min and blocked with TBS-containing 1 % BSA for 1 h.

**1.3 Pro-Q<sup>TM</sup> staining and detection.** Pro-Q<sup>TM</sup> staining was routinely carried out on spotted slides to ensure the consistency and quality of the slides. The slide was washed with H<sub>2</sub>O and stained with Pro-Q<sup>TM</sup> Diamond dye for 1 h at room temperature. Subsequently the slide was destained with a solution of 20 % acetonitrile in sodium acetate (pH = 4) for 0.5 h and visualized with the microarray scanner under the Cy3 channel ( $\lambda_{\text{Ex}}$  = 548 nm;  $\lambda_{\text{Em}}$  = 595 nm).

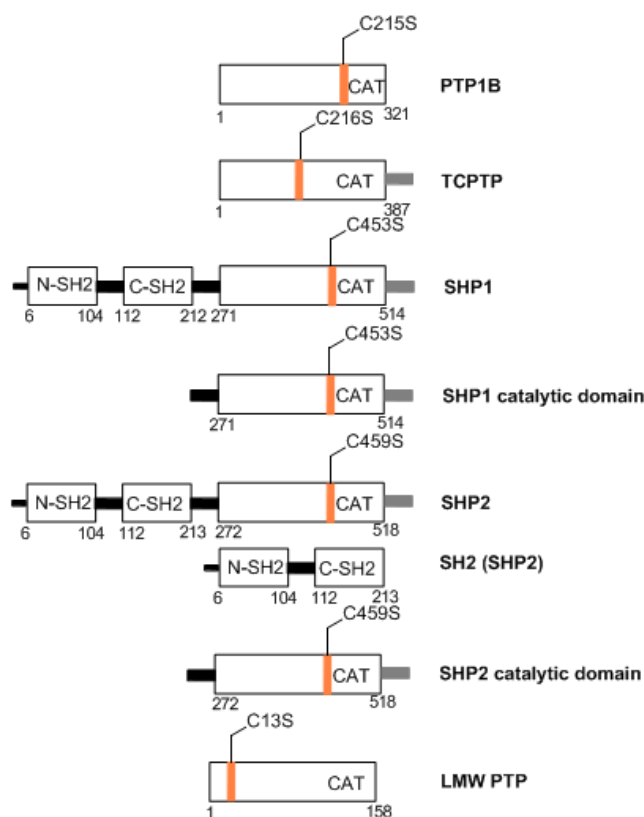
**1.4 Site-directed mutagenesis.** The active site mutants of 5 different PTPs (PTP1B, TCPTP, SHP1, SHP2 and LMWPTP) as well as their domains (Scheme S1) were mutated by using the commercially available site directed mutagenesis Kit. The two catalytic domains of SHP1 and SHP2 were generated by introducing an extra stop codon between the SH2 and the catalytic domain of the corresponding PTPs. Primers were designed to introduce the desired mutation site. Appropriate primer sequences were chosen to ensure that the T<sub>m</sub> melting temperature was above 78 °C. PCR amplification was first carried out to amplify the whole

sequence of gene with the primer containing desired mutation site. The PCR products were run with gel analysis to ensure the desired PCR band. Dpn I enzyme was used to digest the template to remove the false positives. The PCR product after Dpn I digestion was then transformed into top-10 competent cells. DNA sequencing was carried out to confirm the presence of mutation site and proper frame shift of the gene. After verification with DNA sequencing, the gene was transformed into expression host BL21 DE3 for protein expression. In total, 8 proteins/domains were obtained for the study (Scheme S1).

**Table S1.** List of PTPs used in this study.

| PTPs               | Gene symbol | Mutation site | Classification | Used in this study |
|--------------------|-------------|---------------|----------------|--------------------|
| PTP1B              | PTN1        | C215S         | Tyr ppase      | ✓                  |
| TCPTP              | PTPN2       | C216S         | Tyr ppase      | ✓                  |
| SHP1 <sup>1*</sup> | PTPN6       | C453S         | Tyr ppase      | ✓                  |
| SHP2 <sup>*</sup>  | PTPN11      | C459S         | Tyr ppase      | ✓                  |
| LMW PTP            |             | C13S          | Tyr ppase      | ✓                  |

\* Both the full length and the catalytic domain ( $\Delta$ SHP1 and  $\Delta$ SHP2) of the proteins were used in the study.



**Scheme S1.** Schematic representation of the structure of different PTP mutants and domains used in this study.

**1.5 Protein expression and purification.** Overnight cultures were diluted 1:100 in LB media supplemented with 100  $\mu$ g/mL of ampicillin or kanamycin and grown at 37 °C. At OD<sub>600</sub> ~0.6, expression was induced by addition of IPTG (isopropyl-b-D thiogalactopyranoside) and cultures were grown further at either RT or 30 °C overnight. Successful overexpression of the proteins was verified by coomassie blue staining and immunoblot analysis with anti-GST antibody or anti-His antibody (Amersham Biosciences). After cell harvest and lysis (at pH 7.4, 20 mM tris-HCl, 150 mM NaCl) by sonication (6X pulses of 15 s each at half maximal power, on ice), the solution was clarified by centrifugation at 10 000 g, 30 min, 4 °C and affinity purified following vendor's protocols. Fractions containing the desired fusion protein were pooled and

dialysed into a suitable buffer, and stored at -20 °C. Protein concentration was determined using the Bradford protein assay (Bio-Rad). Protein purity was determined by separation on a 12 % SDS-PAGE gel.

**1.6 Protein labeling and screening on the peptide microarray.** Protein samples were minimally labeled with either Cy3 or Cy5 N-hydroxysuccinimide ester (Amersham, GE Healthcare, USA) for 1 h on ice, following the manufacturer's protocols and our previously published procedures.<sup>1</sup> The unreacted dye was quenched with a 10-fold molar excess of hydroxylamine for a further 1 h. The excess dye was further removed by extensive dialysis at 4 °C overnight (Amersham, GE Healthcare, USA). After analysis by SDS-PAGE gel to ensure successful labeling and purity, the labeled protein was reconstituted in a final buffer volume of 80 µl TBS (pH 7.4) containing 1% bovine serum albumin (BSA). In a standard microarray experiment, the labeled protein (1 µM; 80 µL) was applied to the array. In a dose-dependent experiment for K<sub>D</sub> measurements, various concentrations of the protein (i.e. 5000 nM to 50 nM) were applied to different subarrays on the same slide, as previously described.<sup>1</sup> For denaturing experiments (e.g. Fig S4), the labeled protein was first boiled for 5 min, cooled before being applied to the peptide microarray. For dual-color screening experiments, an equal amount of a Cy3-labeled protein and a Cy5-labeled protein was mixed and applied together to the slide.<sup>1</sup> The samples were incubated with the array in a humidified chamber for 1 h at room temperature, before repeated rinses with TBS + 0.05% Tween 20, typically 3 x 10 min washes with gentle shaking. Slides were scanned using an ArrayWoRx microarray scanner installed with the relevant filters (Cy3: λ<sub>ex/em</sub> = 548/595 nm; Cy5: λ<sub>ex/em</sub> = 633/685 nm).

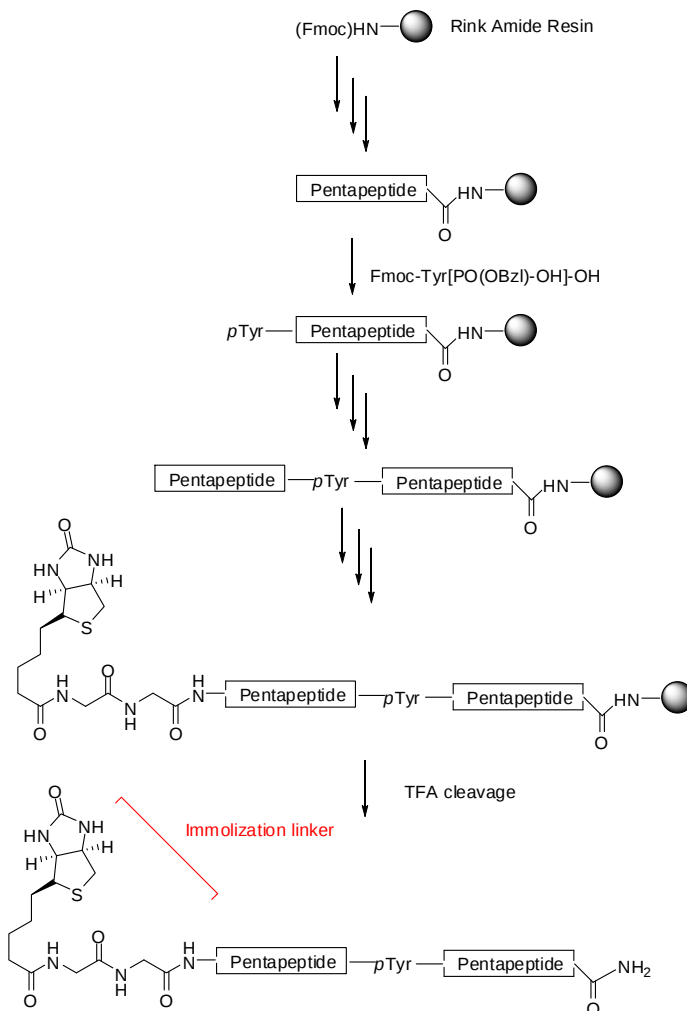
**1.7 Data extraction and analysis.** Microarray data was extracted using the ArrayWoRx software. Values from duplicated points were background subtracted and averaged (Duplicated spots with a standard deviation > 0.2 were rejected). The dataset was presented as colored heatmaps, using the Treeview software (<http://rana.lbl.gov/EisenSoftware.htm>). Other data analysis was carried out with Microsoft Excel. Venn diagrams were generated to identify the potent binders against PTP mutants, using the Venn Diagram Generator (<http://www.pangloss.com/seidel/Protocols/venn.cgi>). For microarray K<sub>D</sub> experiments, the corresponding K<sub>D</sub> was generated by fitting the data to the following equation as previously described,<sup>1</sup> under the assumption that equilibrium is achieved during the incubation period:

$$\text{Observed fluorescence of } x = \frac{[\text{Maximum fluorescence, } x] \times [\text{Protein concentration}]}{K_D + \text{Protein concentration}}$$

**1.8 Microplate PTP enzymatic assay.** Solution-based enzymatic assay was performed as previously described,<sup>2</sup> and measured in 384-well microplates with a total reaction volume of 40 µL. 10 µM solutions of the peptides in Hepes buffer (50 mM Hepes, 100 mM NaCl, 2 mM EDTA, 0.01% Brij, 1 mM DTT, pH 7.0) were incubated with optimum concentrations of PTP1B or TCPTP for different time points (ranging from 5 min, 15 min, 30 min, 60 min to 120 min) at 25 °C. The kinetic data for each peptide was determined in duplicate. The reaction was stopped by addition of malachit green solution containing sulfuric acid. After incubation with the malachit green solution for 10 min, the absorbance of the malachit green-phosphate complex was measured at 650 nm. The microplate data was processed with the software Graphpad Prism v. 4.03 (GraphPad, San Diego, USA) and fitted with the following equation to derive the kinetic data. Abs<sub>Obs</sub> represents the absorbance reading at time x. Abs<sub>Max</sub> represents the fitted constant which should correspond to the maximum absorbance obtained when the enzymatic reaction is complete.

$$\text{Abs}_{\text{Obs}} = \text{Abs}_{\text{Max}} \times (1 - \exp(-k_{\text{obs}} \times \text{time}))$$

## 2. Peptide Synthesis



**Scheme S2.** Schematic representation of peptide synthesis

Peptide synthesis was performed by using standard Fmoc Strategy combined with IRORI™ directed sorting technology, as previously described.<sup>1</sup> Rink amide resin was used as the solid support. Standard HOBt/HBTU/DIEA coupling method was used throughout the whole process. Each microreactor contains around 40 mg of rink amide resin and a unique  $R_f$  tag for sorting application. The resin was swelled in HPLC-grade DMF for 1 h at room temperature. Subsequently the Fmoc group was deprotected by treatment of 20% piperidine for 1 h at room temperature. Following removal of the piperidine, the resin was washed extensively with DMF and DCM. The microreactors were then dried thoroughly under the high vacuum. Next, the microreactors were sorted and distributed into several reaction vessels, and each containing a unique Fmoc amino acid (4.0 eq), preactivated with HBTU/HOBt/DIEA (1/1/2 ratio relative to the amino acid). The resins were swelled in DMF for half an hour before coupling. The coupling reactions were carried out for 8 h at room temperature with shaking. At the end, the microreactors were collected and washed thoroughly with DMF and DCM. Any unreacted resin residue was capped with a solution of  $\text{Ac}_2\text{O}$  (10 eq), DIEA (20 eq) in DCM (200 mL), and the reaction mixture was allowed to react for 2 h at room temperature, followed by extensive wash with DCM and DMF. Subsequently the resin was deprotected with 20% piperidine again and ready for next coupling cycle. Repeat the above cycle until the last amino acid has been coupled. (Biotin)-GG was attached at the N-terminus of peptides for microarray

immobilization.<sup>1</sup> After the whole coupling process was finished, the microreactors were collected, washed thoroughly and dried under high vacuum for 2 h at RT. The microreactors were then decoded and cleaved under 95% TFA, 2.5% TIS, 2.5% H<sub>2</sub>O for 4 h at room temperature. For those peptides containing cysteine or methionine residue, the peptides were cleaved from the resin by using 94.5%TFA, 2.5% EDT, 2.5% H<sub>2</sub>O and 1% TIS. Following prolonged concentration *in vacuo* until >80% of cleavage cocktail was removed. Cold ether (chilled to -20 °C) was added to the liquid residue to precipitate the peptides. The peptides were allowed to precipitate at -20 °C for overnight. At the end, the ether layer was decanted and the precipitates were dried thoroughly *in vacuo*. This process was repeated for a couple more times. The resulting peptide solids were dissolved in 1 ml DMSO and stored at -20 °C. LCMS was performed, as previously described,<sup>1</sup> to ensure the peptides were of correct mass and sufficient purity for subsequent microarray experiments.

**Table S2.** List of 144 peptides used in this study. These PTP putative substrates are derived from (1) known literature; (2) human protein reference database at [www.hprd.org](http://www.hprd.org), and (3) <http://www.phosphosite.org>. The table below shows the identity and sequence of each peptide, the molecular weight as determined by LCMS, the corresponding PTP targeted and its original protein source.

| NO | ID  | Peptide Sequence | MW <sup>a</sup> | Enzyme Targeted <sup>b</sup> | Oroginal Protein Source (pTyr site) |
|----|-----|------------------|-----------------|------------------------------|-------------------------------------|
| 1  | A01 | AEKPFpYVNVEF     | 1762            | PTP1B                        | BCR (177)                           |
| 2  | A02 | AEMTGpYVVTRW     | 1732            |                              | Mitogen kinase                      |
| 3  | A03 | AENAEpYLRVAP     | 1652            | SHP1                         | EGF receptor (1197)                 |
| 4  | A04 | DEELHpYASLNF     | 1757            | SHP1                         | CD33 (340)                          |
| 5  | A05 | DEGIHpYSELIQ     | 1723            | SHP1                         | SIGLEC2 (822)                       |
| 6  | A06 | DEKVDpYVQVDK     | 1757            | SHP1, SHP2                   | Gab2 (643)                          |
| 7  | A07 | DERVDpYVVVDQ     | 1756            | SHP2                         | Gab1 (689)                          |
| 8  | A08 | DESVDpYVPMLD     | 1702            | SHP2, TCPTP                  | PDGF (751)                          |
| 9  | A09 | DKQVEpYLDLDL     | 1770            | SHP2                         | GAB1 (657)                          |
| 10 | A10 | DSGGFpYITSRT     | 1623            |                              | Src-1                               |
| 11 | A11 | DTETVpYSEVRK     | 1746            | SHP1                         | CD31 (713)                          |
| 12 | A12 | EANSHpYGHND      | 1678            | SHP1                         | CD31 (663)                          |
| 13 | A13 | EDEDYpYKASVT     | 1739            | Type 12                      |                                     |
| 14 | A14 | EDGGVpYSSSGL     | 1489            | Photooncogene FER kinase     |                                     |
| 15 | A15 | EDGISpYTTLRF     | 1721            | SHP1                         | SIGLEC2 (762)                       |
| 16 | A16 | EDSTYpYKASKG     | 1668            |                              | Fak chick kinase                    |
| 17 | A17 | EDTLTpYADLDM     | 1706            | SHP2                         | SIRP (470)                          |
| 18 | A18 | EGVATpYAAAVL     | 1484            | SHP1                         | CATENIN (654)                       |
| 19 | A19 | EGDNDpYIPLP      | 1665            | TCPTP                        | PDGF (1021)                         |
| 20 | A20 | ENGLNpYIDLDL     | 1698            | SHP2, PTP1B                  | IRS1 (1179)                         |
| 21 | A21 | EPNVSpYICSR      | 1750            |                              | Kinase gsk3                         |
| 22 | A22 | ESDGSpYQKPSY     | 1680            | SHP2                         | SELECTIN E (603)                    |
| 23 | A23 | ESDGGpYMDMSK     | 1638            | SHP2                         | PDGF (740)                          |
| 24 | A24 | ETDKEpYTVKD      | 1810            | TCPTP                        | JANUS KINASE1 (1022)                |
| 25 | B01 | FEEDDpYESPND     | 1779            | SHP1                         | SLP76 (113)                         |
| 26 | B02 | FGMTRpYVLDDE     | 1765            | Tyrosine kinase TXK          |                                     |
| 27 | B03 | FLFNMpYLTRER     | 1909            | SHP1                         | HOAX1 (343)                         |
| 28 | B04 | FMMTPpYVTRY      | 1827            |                              | JNK-2 kinase                        |
| 29 | B05 | GFLTEpYVATRW     | 1762            | PTP1B                        | ERK2 (187)                          |
| 30 | B06 | GNNYVpYIDPTQ     | 1703            | SHP1                         | KIT (570)                           |
| 31 | B07 | GSAAPpYLKTKF     | 1602            | TCPTP                        | STAT3 (705)                         |
| 32 | B08 | GWMEDpYDYVHL     | 1847            |                              | P130cas                             |
| 33 | B09 | GWMMVpYTSKDT     | 1744            |                              | protein kinase c                    |
| 34 | B10 | HNSALpYSQVQK     | 1694            |                              | p62dok                              |

|    |     |               |      |                             |                             |
|----|-----|---------------|------|-----------------------------|-----------------------------|
| 35 | B11 | HRQLNpYIQVDL  | 1818 | SHP2                        | FRS2 (436)                  |
| 36 | B12 | IEDNEpYTAREG  | 1716 | SHP1                        | LYN (397)                   |
| 37 | B13 | IEDEDpYYKASV  | 1751 | Type 12                     |                             |
| 38 | B14 | IEDNEpYTARQG  | 1715 | SHP1,type 18,TCPTP          | C-SRC (419)                 |
| 39 | B15 | IESDIpYAEIPD  | 1684 | Type 12                     |                             |
| 40 | B16 | IESSNpYMAPYD  | 1709 | PTP1B,TCPTP                 | PDGF (771)                  |
| 41 | B17 | IRYHRpYHGSA   | 1835 | Protooncogene kinase, PIM 1 |                             |
| 42 | B18 | IYETDpYYRKGG  | 1784 | TCPTP                       | INSULIN (1189)              |
| 43 | B19 | KAVDGpYVKPQI  | 1637 | SHP2, PTP1B                 | STAT5B (699)                |
| 44 | B20 | KDRMSpYHVRSH  | 1835 |                             | Myc Zinc protein            |
| 45 | B21 | KKRCpYTKHQT   | 1809 | SHP1                        | HOAX10 (326)                |
| 46 | B22 | KVVALpYDYMPM  | 1749 |                             | BTK kinase                  |
| 47 | B23 | LISSDpYELLSD  | 1674 | TCPTP                       | JAK3 (785)                  |
| 48 | B24 | LNSDGpYTPEPA  | 1583 | LMW                         | ZAP70 (292)                 |
| 49 | C01 | LNSKGpYTKSID  | 1645 |                             | Erk kinase                  |
| 50 | C02 | LPPEGpYVVVVK  | 1619 | SHP2                        | PTK2B (906)                 |
| 51 | C03 | MDTSVpYESPYS  | 1698 | Tyrosine kinase Zap 70      |                             |
| 52 | C04 | MKDEEpYEQMVK  | 1849 | type 13                     |                             |
| 53 | C05 | MTGDTpYTAHAG  | 1544 | type 12                     |                             |
| 54 | C06 | NNHTEpYASIQT  | 1697 | SHP1, SHP2                  | SIRP (453)                  |
| 55 | C07 | NQSSGpYRYGTD  | 1667 |                             | Fyn kinase                  |
| 56 | C08 | NSDVQpYTEVQV  | 1701 | SHP1, SHP2                  | CD31 (690)                  |
| 57 | C09 | NSKRDpYTGCS   | 1651 | SHP2                        | MYELIN (200)                |
| 58 | C10 | NVVLPYDLLLE   | 1707 | PTP1B                       | ESTROGEN RECEPTOR (537)     |
| 59 | C11 | PEGLNpYACLTH  | 1637 | SHP1                        | ROS1 (2334)                 |
| 60 | C12 | PEGHEpYpYRVRE | 1934 | PTP1B                       | TYK2 (1054)                 |
| 61 | C13 | PEQDEpYDIPRH  | 1818 |                             | P130cas                     |
| 62 | C14 | PGSLEpYLCLPA  | 1582 | SHP1, SHP2                  | INTERLUKIN 3 RECEPTOR (628) |
| 63 | C15 | PKGTGpYIKTEL  | 1626 | SHP2,TCPTP                  | STAT1 (701)                 |
| 64 | C16 | PLDKDpYpYVVRE | 1896 | TCPTP                       | JAK3 (981)                  |
| 65 | C17 | PPDHQpYYNDFP  | 1812 | TCPTP                       | SHC (349)                   |
| 66 | C18 | PQDKEpYpYKVKE | 1846 | SHP2,PTP1B                  | JANUS KINASE2 (1007)        |
| 67 | C19 | PSFSEpYASVQV  | 1633 | SHP2                        | SIRP (496)                  |
| 68 | C20 | PSTRDpYpYIQRE | 1813 |                             | Fak kinase                  |
| 69 | C21 | PQDKEpYpYKVKE | 1926 |                             | JAK kinase                  |
| 70 | C22 | QGPVI pYAQLDH | 1660 | SHP2                        | MYELIN (241)                |
| 71 | C23 | QKQPIpYIVMEL  | 1781 |                             | FES kinase                  |
| 72 | C24 | QQQEVpYGMMPR  | 1786 | LMW                         | SPECTRIN (1176)             |
| 73 | D01 | RNEGVpYTAIAV  | 1612 | Type 12                     |                             |
| 74 | D02 | REGLNpYpYMLAT | 1686 | SHP1                        | ROS1 (2274)                 |
| 75 | D03 | SAEPQpYQPGDQ  | 1639 | PHotooncogene FGR kinase    |                             |
| 76 | D04 | SDDVRpYVNAFK  | 1733 | SHP2                        | VEGF (1213)                 |
| 77 | D05 | SDGHEpYpYVDP  | 1714 | TCPTP                       | PDGF (579)                  |
| 78 | D06 | SEEPipYIVTEY  | 1762 | SHP1                        | C-SRC (338)                 |
| 79 | D07 | SESVVpYADIRK  | 1686 | SHP2                        | MYELIN (263)                |
| 80 | D08 | SETDDpYAEIID  | 1690 | Type 12                     |                             |
| 81 | D09 | SPPALpYAEPLD  | 1592 |                             | P62dok                      |
| 82 | D10 | SSNPEpYLSASD  | 1589 | TCPTP                       | INSULIN (999)               |

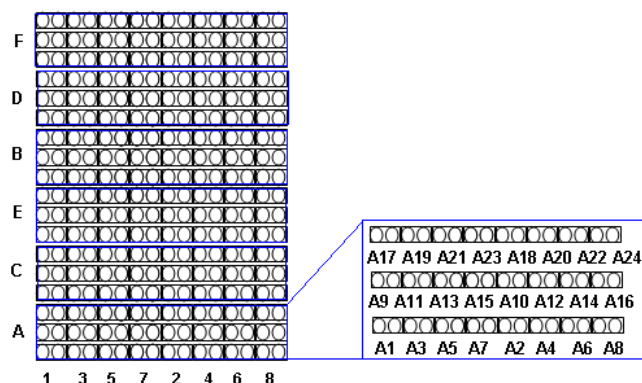
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|-----|-----|---------------|------|-------------|-----------------------------------|
| 83  | D11 | SSSSEpYGSVSP  | 1505 |             | Mitogen kinase                    |
| 84  | D12 | SSTVQpYSTVVH  | 1627 | SHP2        | INTERLUKIN 6 RECEPTOR (759)       |
| 85  | D13 | STEPQpYQPGEN  | 1669 | SHP1,PTP1B  | C-SRC (530)                       |
| 86  | D14 | TDKEYpYTVKDD  | 1796 | TCPTP       | JANUS KINASE 1 (1023)             |
| 87  | D15 | TGGSVpYTEDND  | 1576 | Type 3      |                                   |
| 88  | D16 | TNDITpYADLNL  | 1672 | SHP2        | SIRP (429)                        |
| 89  | D17 | TSKLI pYDFIED | 1763 | Type 12     |                                   |
| 90  | D18 | TSSVLpYTAVQP  | 1585 | SHP2        | PDGF (1009)                       |
| 91  | D19 | VDADEpYLIPQQ  | 1710 | SHP2, PTP1B | EGFR (1016)                       |
| 92  | D20 | VDTPHpYPRWIT  | 1804 | PKC Kinase  |                                   |
| 93  | D21 | VNTTLpYEKFTY  | 1798 | SHP2        | TEK KINASE (1108)                 |
| 94  | D22 | VYESPpYSDPEE  | 1734 |             | Tyrosine kinase zap-70            |
| 95  | D23 | YETDYpYRKGGK  | 1799 | PTP1B,TCPTP | INSULIN (1190)                    |
| 96  | D24 | YFMTEpYVATRW  | 1886 |             | Mitogen kinase                    |
| 97  | E01 | AEGSApYEEVPT  | 1572 |             | PLC-r                             |
| 98  | E02 | AENPEpYLSEFS  | 1705 |             | Erb2 receptor                     |
| 99  | E03 | FDNLYpYWDQDP  | 1895 |             | Erb2 receptor                     |
| 100 | E04 | AFGTVpYKGIWI  | 1674 |             | Erb2 receptor                     |
| 101 | E05 | AFQFSpYTAVFG  | 1657 |             | CAAX Protease                     |
| 102 | E06 | AKIQDpYHILTR  | 1777 |             | Jak2                              |
| 103 | E07 | APAEMpYDIMKT  | 1689 |             | Stem cell growth factor           |
| 104 | E08 | ASEQGpYEEMRA  | 1690 |             | Erb3                              |
| 105 | E09 | ATVGHpYTAVQN  | 1580 |             | Cyclin kinase activator           |
| 106 | E10 | AYRQLpYLNPKE  | 1741 |             | Chromosomal associate protein     |
| 107 | E11 | CPEKVpYELMRA  | 1758 |             | Abl 1                             |
| 108 | E12 | DINSLpYDVSRM  | 1732 |             | PLC-r                             |
| 109 | E13 | DRFIQpYANPAF  | 1761 |             | Camp Phosphodiesterase            |
| 110 | E14 | DVSRMpYVDPSE  | 1717 |             | PLC-r                             |
| 111 | E15 | EDIKSpYTVRQ   | 1821 | PTP1B       |                                   |
| 112 | E16 | EEIRFpYQLGEE  | 1832 |             | Potassium gate                    |
| 113 | E17 | EKIQDpYEKMPE  | 1829 |             | IL-1                              |
| 114 | E18 | ELGYEpYMDVGS  | 1682 |             | Erb-3                             |
| 115 | E19 | AELEFpYMDYEA  | 1800 | PTP1B       | Alanine scanning                  |
| 116 | E20 | FGAKPpYDGIPA  | 1555 |             | Erb2 receptor                     |
| 117 | E21 | GPQDIpYDVPPV  | 1619 |             | P130cas                           |
| 118 | E22 | GQSEpYGNITY   | 1680 | PTN6        |                                   |
| 119 | E23 | GRETIpYPNASL  | 1640 |             | Carcinoembryonic antigen          |
| 120 | E24 | IGEGTpYGTVFK  | 1591 |             | Cell division kinase 5            |
| 121 | F01 | IYIHRpYENVSI  | 1826 |             | ATP cyclase                       |
| 122 | F02 | KAEDepYVNEPL  | 1726 |             | Erb2 receptor                     |
| 123 | F03 | KPKQEpYLNPE   | 1764 |             | Erb4                              |
| 124 | F04 | LARDMpYDKEYY  | 1886 |             | Met growth factor                 |
| 125 | F05 | LDSTFpYRSLLE  | 1763 |             | Erb2 receptor                     |
| 126 | F06 | LGQRipYQYIQS  | 1788 |             | Dual specificity regulated kinase |
| 127 | F07 | LLANApYIYVVQ  | 1686 |             | Neural cell adhesion              |
| 128 | F08 | LMGHEpYMEMKN  | 1802 |             | Cell division cycle protein 23    |
| 129 | F09 | LNKQGpYKCRQC  | 1760 |             | Protein kinase C                  |
| 130 | F10 | NKPTVpYGVSPN  | 1595 |             | Abl                               |

|     |     |               |      |                                             |
|-----|-----|---------------|------|---------------------------------------------|
| 131 | F11 | PPDHQpYYNDFP  | 1812 | SHC transforming protein                    |
| 132 | F12 | PEDTFpYFDPEF  | 1826 | ISPK-1 Kinase                               |
| 133 | F13 | PEPGPpY AQPSV | 1561 | Adaptor crk                                 |
| 134 | F14 | RHDSGpYEVHHQ  | 1784 | Alzheimer's disease                         |
| 135 | F15 | RNPGFpYVEANP  | 1683 | PLC-r                                       |
| 136 | F16 | SADHLpYVNVSE  | 1653 | Tumor necrosis factor                       |
| 137 | F17 | SSDPTpYTSSLG  | 1534 | Tyrosine kinase receptor                    |
| 138 | F18 | EEEPVpYEAEPE  | 1728 | Hematopoietic lineage cell specific protein |
| 139 | F19 | STKYFpYKQNGR  | 1811 | Erb4                                        |
| 140 | F20 | TAEPDpYGALYE  | 1648 | PLC-r                                       |
| 141 | F21 | VCAERpYSQEVF  | 1750 | Apoptosis Protein cd27                      |
| 142 | F22 | VDSSLpYNLPRS  | 1670 | Grb2 associated                             |
| 143 | F23 | VSSTHpYYLLPE  | 1728 | Activated cdc42 kinase                      |
| 144 | F24 | VVIALpYDYQTN  | 1718 | T cell specific kinase                      |

<sup>a</sup>Most peptides (>98%) were shown to possess the expected molecular weight and have sufficient purity (>85%) as judged by LCMS analysis.

<sup>b</sup>Obtained from literature and various databases ([www.hprd.org](http://www.hprd.org); [www.phosphosite.org/](http://www.phosphosite.org/))

### 3. Results and Discussion



**Figure S1.** The spotting format of all the microarrays used in this study, unless otherwise specified.

(a)

PTP1B  
TCPTP  
SHP1 - - MVRWFH RDL SGLDAETLL KGRGVHGSFLARPSRKNQGD FSLSVRVGDQVTHIRIQNSG 58  
SHP2 MTSRRWFHPNITGVEAENLLL TRGVDSFLARPSKSNPGDFTLSVRRNGAVTHIKIQNTG 60  
LMW

PTP1B  
TCPTP  
SHP1 DFYDLYGG EK FATL TELVEYYTQQGVLDQDGTIIHLKYPLNCSDPTSERWYHGHMSGG118  
SHP2 DYYDLYGG EK FATLAELVQYMEHHGQLKEKNGDVIELKYPLNCADPTSERWFHGHLSGK120  
LMW

PTP1B  
TCPTP  
SHP1 QAETLLQAKGEPWTFVLVRESLSQPGDFVLSVLSQDPKAGPGS-PLRVTHIKVMCEGGRYT177  
SHP2 EAEKLLTEKGKHGSFLVRESQSHPGDFVLSVTRTGDDKGESNDGKSKVTHVMIRCQELKYD180  
LMW

PTP1B  
TCPTP  
SHP1 VGGLETFD SLTDLVEHFKKTGIEEASGAFVYLRQPYATRVNAADIENRVLELNKKQSE237  
SHP2 VGGGERFDSL TDLVEHYKKNPMVETLGTVLQLKQPLNTRINAAEIESRVRELSKLAETT240  
LMW

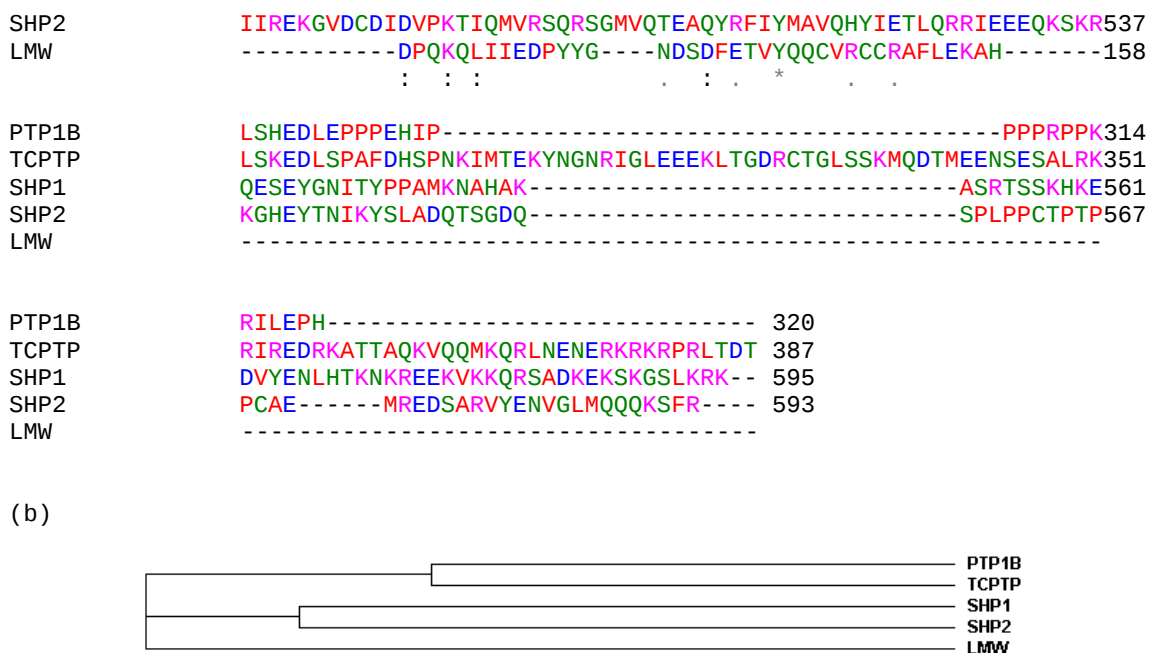
PTP1B  
TCPTP  
SHP1 ---SGSWAAIYQDIRHEASDFPCR-VAKLPKNKNRNRYRDVSPFDHSRIKLHQED--- 63  
SHP2 ---QRRWQPLYLEIRNESH DYPHR-VAKFPENNRNRYRDVSPYDHSRVKLQNAE--- 65  
LMW DTAKAGFWEEFESLQKQEVKNLHQRLEGQRPENKGNRYKNILPFDHSRVILQGGRDSNIP297  
DKVKQGFWEEFETLQQQECKLLYSRKEGQRQENKNKNRYKNILPFDHTRVVLHDGDPNEP300

PTP1B  
TCPTP  
SHP1 -NDYINASLIKMEEA-----QRSYILTQGPLNTCGHFWMVWEQKSRGVVMLNRVM114  
SHP2 -NDYINASLV D IEEA-----QRSYILTQGPLNTCCHFWMVWQKTKAVVMLNRIV116  
LMW GSDYINANYIKNQLLGPDEN---AKTYIASQGCL EATVNDFWQMAWQENS RVIVMTTREV354  
VSDYINANIIMPEFETKCNNSKPKKSYIATQGCLQNTVNDFWRMVFQENS RVIVMTTKEV360  
MAEQATKSVLFCVCLGNICRSPIAEAVFRKLVT DQNI 36  
: : : \* : : : :

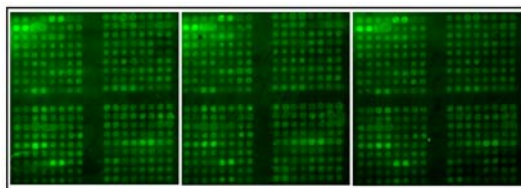
PTP1B  
TCPTP  
SHP1 EKGS LKCAQYWPQKEEKEMIFEDTNLKLTLISEDIKSYYTVRQLELENLTQ-ETREILH173  
SHP2 EKESVKCAQYWP-TDDQEMLFKETGFSVKLLS E DVKSYTVHLLQLENINSG-ETRTISH174  
LMW EKGRNKCVPYWP---EVGMQRAYGPYSVTNCGEHDTEYKLR TLQVSPLDNGDLIREIWH411  
ERGKSKCVKYWP---DEYALKEYGVMVRNVKESAHDYTLRELKLSKVGGQNTERTVWQ417  
SEN-----WVIDSGAVSDWNVGRSPDPR-AVSCLRNHGIHTAHKARQITK 80  
\* \* : \* : :

PTP1B  
TCPTP  
SHP1 FHYTTWPDFGVPE SPASFLNFLFKVRESGSLSP EHGPPVVHCSAGIGRSGTFCLADTCLL233  
SHP2 FHYTTWPDFGVPE SPASFLNFLFKVRESGSLNPDHGPAV IHCSAGIGRSGTFSLVDTCLV234  
LMW YQYLSWPDHGVPE SPGGVLSFLDQINQRQESLPHAGPIIVHCSAGIGRTGTIIVIDMLME471  
YHFRTPD HGVPSDPGGVLD FLEEVHHKQESIMDAGPVVHCSAGIGRTGT FIVIDILID477  
EDFATF-DYILCMDES NLRDLNRKSNQ-----VKTC KAKIELLGSY-----120  
: : : \* : : : : : \* \* \* \* :

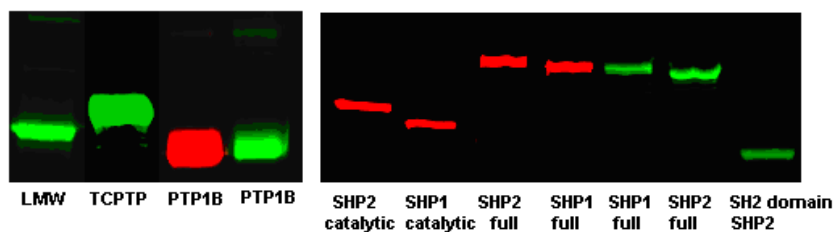
PTP1B  
TCPTP  
SHP1 LMDKRKDPSSVDIKKVLL EMRKFRMGLIQTADQLRFSYLAVIEGAKFIMG DSSVQDQWKE293  
LMEKGDD---INIKQVLLNMRKYRMGLIQT PDQLRFSYMAIEGAKCIKG DSSIQRWKE291  
NISTKGLDCDI IQTKI QMVRAQRSGMVQTEAQYKFIYVAIAQFIETT KKKLEVLQSQKG531



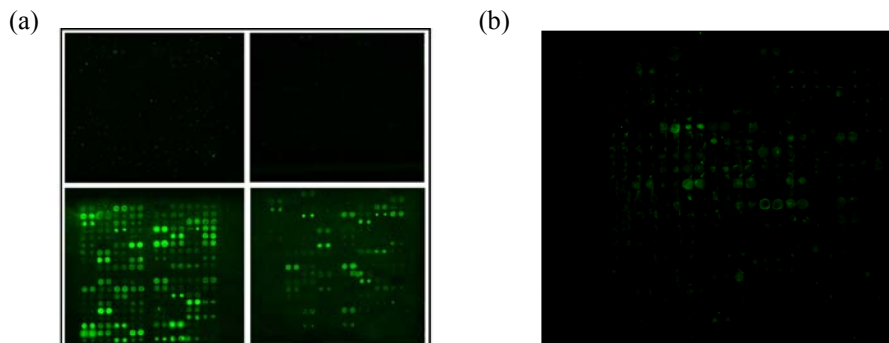
**Figure S2.** The sequence homology analysis of the 5 different PTPs used in the study.  
(a) Sequence alignment. (b) Cluster analysis.



**Figure S3.** The Pro-Q™ images of the 144-member pTyr peptide library, spotted on three separate microarrays, indicating a high degree of slide-to-slide and (duplicated) spot-to-spot reproducibility of our microarrays. Different peptides show varied degrees of Pro-Q binding which had been observed previously.<sup>1a</sup>



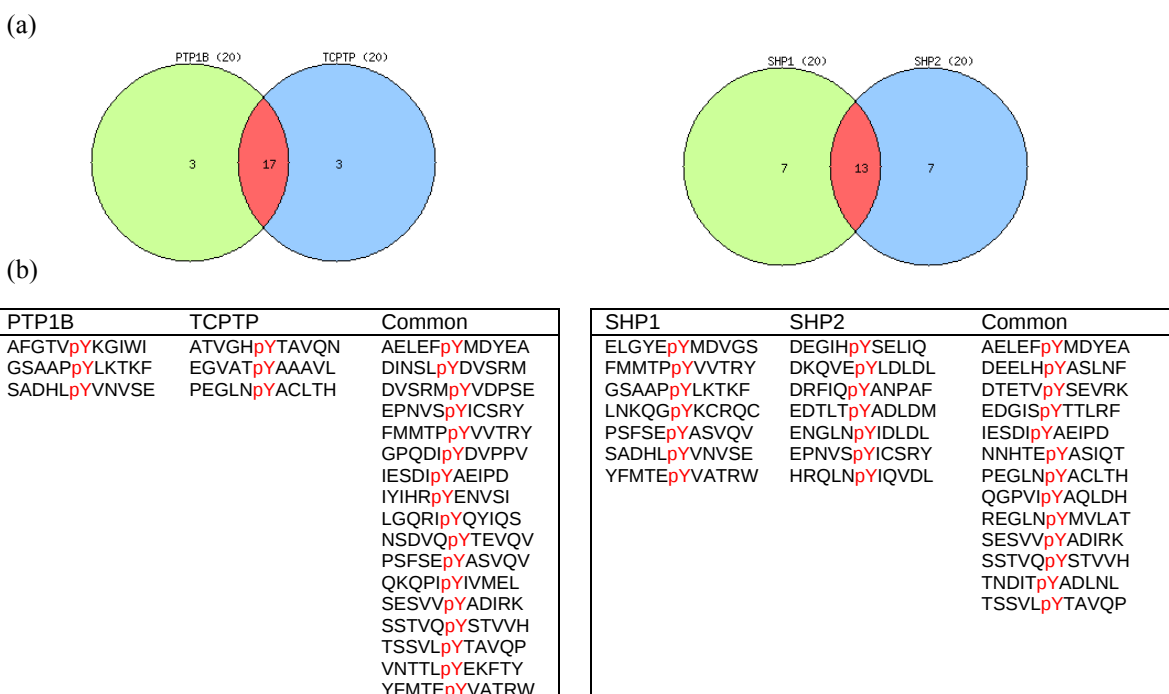
**Figure S4.** Fluorescence gel images of the dye-labeled PTP mutants/domains used in this study. Cy3- and Cy-5 labeled proteins were false-colored in Green and Red, respectively.



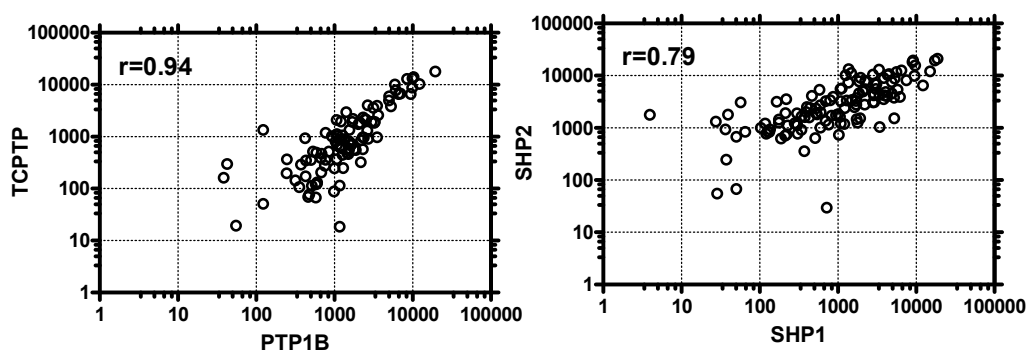
**Figure S5.** The 144-member pTyr peptide microarray is shown to bind to PTP trapping mutants specifically. (a) Denatured (top) and native (bottom) PTP mutants (Left: full-length SHP1; Right: PTP1B; 1  $\mu$ M each) were applied to the peptide microarray and imaged. Results indicate that the ability of PTP mutants binding to the pTyr peptide on the microarray was completely abolished by heat denaturation. (b) 14-3-3 protein (a well-known pSer/Thr peptide binding protein), which is previously shown to binding to a p-Ser/Thr peptide microarray,<sup>1</sup> didn't show measurable specific binding to any of the p-Tyr peptides on the microarray, indicating our pTyr peptide microarray is highly specific for screening of PTP mutants. One  $\mu$ M of each protein was used in the experiment.

### 3.1. Single-point microarray fingerprinting experiments.

**3.1.1 Top-20 analysis based on single-point fingerprinting.** The results were obtained from the images shown in Figure 2 in the maintext, and summarized in the tables in the supplementary excel file "SI2\_Sun.xls". The top-20 scorers based on the absolute fluorescence intensity of the spots were analyzed by Venn Diagram.

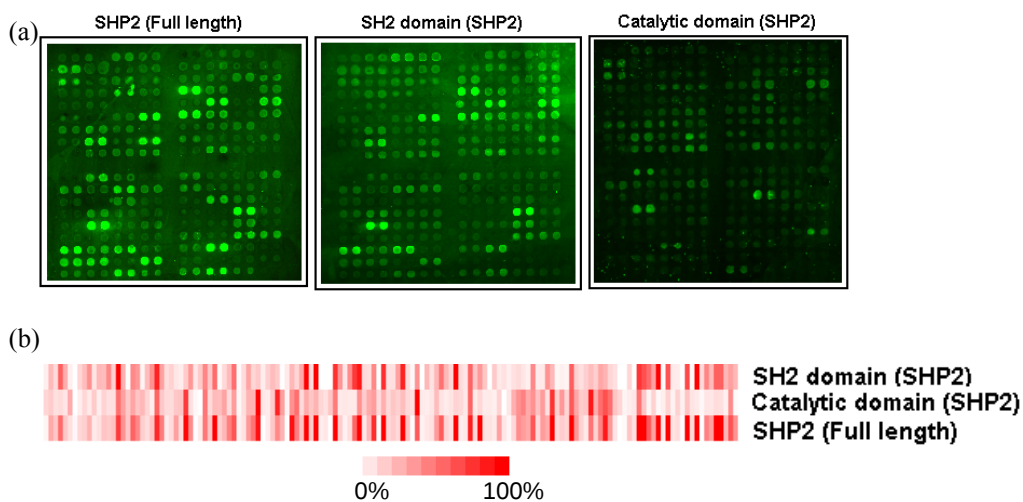


(c)



**Figure S6.** (a) The Venn diagrams comparing the top-20 sequences (based on single-point data in SI2\_Sun.xls) for PTP1B/TCPTP and full-length SHP1/SHP2. (b) Specific peptide sequences obtained from (a). (c) The scatter plot comparing the “fingerprint” homology of PTP1B/TCPTP and SHP1/SHP2 pairs. The single-point data obtained for all 144 peptides were used (SI2\_Sun.xls).

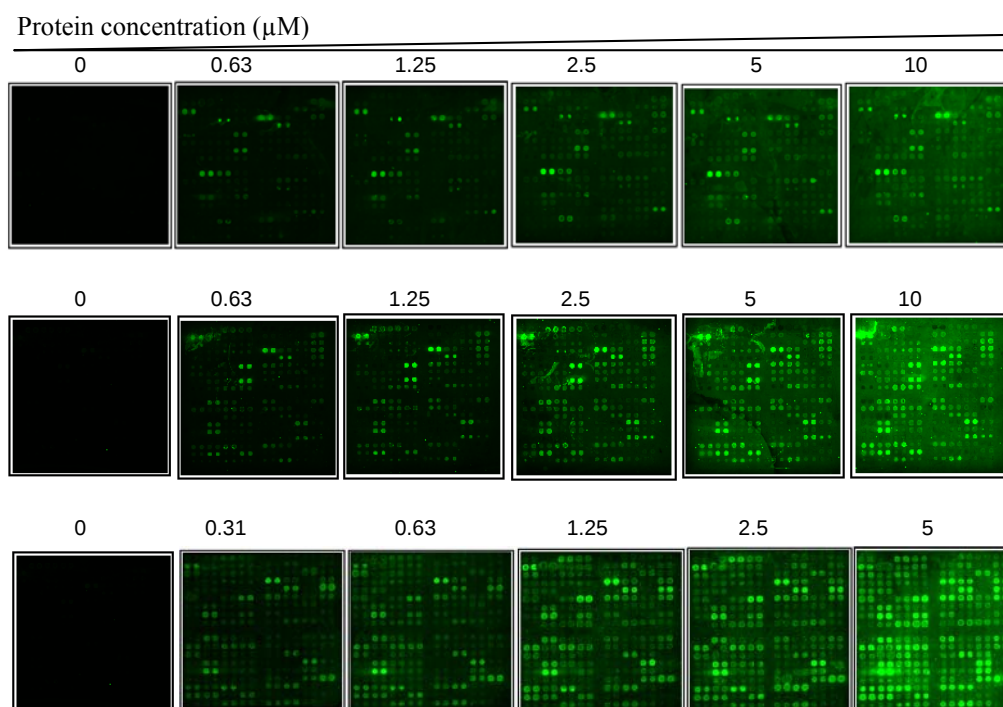
### 3.1.2. Fingerprints of different SHP2 mutants (full-length, catalytic domain and SH2 domain).



**Figure S7.** (a) Microarray images of the 144 peptides binding to full-length SHP (left), SH2 domain of SHP2 (middle) and catalytic domain of SHP2 (right). All proteins used were 1  $\mu$ M. (b) The treeview graph of (a). It indicates the binding of the SH2 domain and catalytic domain combine to contribute to the full-length SHP2 protein binding in our peptide microarray.

### 3.2. Microarray-based $K_D$ experiments.

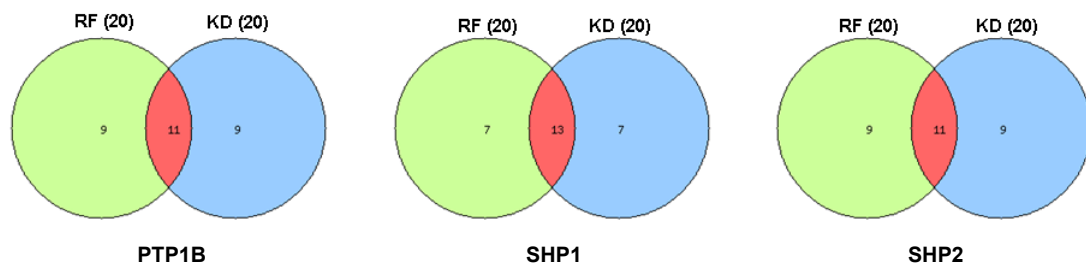
**3.2.1. Microarray  $K_D$  determination.** Complete  $K_D$  obtained for three PTPs (PTP1B, SHP1 and SHP2) were shown in the supplementary excel file "SI2\_Sun.xls". Microarray  $K_D$  experiments for TCPTP failed to give satisfactory results due to high degree of nonspecific binding at high protein concentrations ( $> 1 \mu\text{M}$ ). Fitted covers were shown in Fig S11. Selected  $K_D$  were summarized in Tables S5 & S6.



**Figure S8.** Microarray images of concentration-dependent experiments with PTP1B (top), SHP1 (middle) and SHP2 (bottom). Data were extracted and fitted, assuming a saturation binding model, to derive the corresponding  $K_D$ . Fitted curves were shown in Fig S11 and summarized in the supplementary excel file "SI2\_Sun.xls".

**Table S3.** Comparison between the top-20 peptides obtained from the single-point data (Single-point data in “SI2\_Sun.xls”) and those obtained from  $K_D$  experiments ((XXX  $K_D$  in “SI2\_Sun.xls”) for three PTPs (PTP1B, SHP1 and SHP2). Overlapping sequences from each protein experiment were shaded with the same color.

| Ranking | PTP1B                     |                    | SHP1                      |                     | SHP2                      |                     |
|---------|---------------------------|--------------------|---------------------------|---------------------|---------------------------|---------------------|
|         | Single-point fluorescence | $K_D$              | Single-point fluorescence | $K_D$               | Single-point fluorescence | $K_D$               |
| 1       | AELEFpYMDYEA (E19)        | IYIHRpYENVSI (F01) | TSSVLpYTAVQP (D18)        | IESDlpYAEIPD (B15)  | TSSVLpYTAVQP (D18)        | TSSVLpYTAVQP (D18)  |
| 2       | TSSVLpYTAVQP (D18)        | NNHTEpYASIQT (C06) | SESVVpYADIRK (D07)        | SADHLpYVNVSE (F16)  | QGPVlpYAQLDH (C22)        | TNDITpYADLNL (D16)  |
| 3       | VNTTLpYEKFTY (D21)        | PSFSEpYASVQV (C19) | IESDlpYAEIPD (B15)        | TSSVLpYTAVQP (D18)  | SESVVpYADIRK (D07)        | SSTVQpYSTVVH (D12)  |
| 4       | IESDlpYAEIPD (B15)        | SESVVpYADIRK (D07) | LNKQgpYKCRQC (F09)        | SESVVpYADIRK (D07)  | PEGLNpYACLTH (C11)        | SESVVpYADIRK (D07)  |
| 5       | GPQDlpYDVPPV (E21)        | EGVATpYAAAVL (A18) | SSTVQpYSTVVH (D12)        | AELEFpYMDYEA (E19)  | SSTVQpYSTVVH (D12)        | IYIHRpYENVSI (F01)  |
| 6       | NSDQpYTEVQV (C08)         | TSSVLpYTAVQP (D18) | DTETVpYSEVRK (A11)        | SSTVQpYSTVVH (D12)  | ENGLNpYIDL DL (A20)       | ENGLNpYIDL DL (A20) |
| 7       | IYIHRpYENVSI (F01)        | LMGHEpYMEMKN (F08) | QGPVlpYAQLDH (C22)        | SETDDpYAEIID (D08)  | EDTLTpYADLDM (A17)        | QGPVlpYAQLDH (C22)  |
| 8       | SESVVpYADIRK (D07)        | LNKQgpYKCRQC (F09) | PEGLNpYACLTH (C11)        | ELGYEpYMDVGS (E18)  | EDGISpYTTLRF (A15)        | EDIKSpYTVRQ (E15)   |
| 9       | EPNVSpYICSRy (A21)        | AELEFpYMDYEA (E19) | AELEFpYMDYEA (E19)        | PSFSEpYASVQV (C19)  | IESDlpYAEIPD (B15)        | HRQLNpYIQVDL (B11)  |
| 10      | SSTVQpYSTVVH (D12)        | GPQDlpYDVPPV (E21) | EDGISpYTTLRF (A15)        | EGVATpYAAAVL (A18)  | REGLNpYMLAT (D02)         | PEGLNpYACLTH (C11)  |
| 11      | QKQPlpYIVMEL (C23)        | VNTTLpYEKFTY (D21) | YFMTEpYVATRW (D24)        | VNTTLpYEKFTY (D21)  | HRQLNpYIQVDL (B11)        | LGQRlpYQYIQS (F06)  |
| 12      | YFMTEpYVATRW (D24)        | IESDlpYAEIPD (B15) | PSFSEpYASVQV (C19)        | DTETVpYSEVRK (A11)  | DEELHpYASLNF (A04)        | YFMTEpYVATRW (D24)  |
| 13      | DINSLpYDVSRM (E12)        | ATVGHpYTAVQN (E09) | REGLNpYMLAT (D02)         | IGEGTpYGT VFK (E24) | EPNVSpYICSRy (A21)        | FLFNMpYLTRER (B03)  |
| 14      | DVSRMpYVDPSE (E14)        | QGPVlpYAQLDH (C22) | NNHTEpYASIQT (C06)        | GPQDlpYDVPPV (E21)  | TNDITpYADLNL (D16)        | AFGTVpYKGIWI (E04)  |
| 15      | FMMPpYVVTRY (B04)         | YFMTEpYVATRW (D24) | SADHLpYVNVSE (F16)        | YFMTEpYVATRW (D24)  | DEGIHpYSEL IQ (A05)       | DEELHpYASLNF (A04)  |
| 16      | PSFSEpYASVQV (C19)        | VDTPHpYPRWIT (D20) | ELGYEpYMDVGS (E18)        | DEELHpYASLNF (A04)  | DTETVpYSEVRK (A11)        | LMGHEpYMEMKN (F08)  |
| 17      | GSAAPpYLKTKF (B07)        | KVVALpYDYMPM (B22) | GSAAPpYLKTKF (B07)        | LMGHEpYMEMKN (F08)  | DKQVEpYLDL DL (A09)       | EDGISpYTTLRF (A15)  |
| 18      | SADHLpYVNVSE (F16)        | DINSLpYDVSRM (E12) | FMMPpYVVTRY (B04)         | QGPVlpYAQLDH (C22)  | DRFIQpYANPAF (E13)        | KAVDGpYVKPQI (B19)  |
| 19      | AFGTVpYKGIWI (E04)        | GWMEpYDYVHL (I)    | DEELHpYASLNF (A04)        | FMMPpYVVTRY (B04)   | NNHTEpYASIQT (C06)        | REGLNpYMLAT (D02)   |
| 20      | LGQRlpYQYIQS (F06)        | QKQPlpYIVMEL (I)   | TNDITpYADLNL (D16)        | HRQLNpYIQVDL (B11)  | AELEFpYMDYEA (E19)        | KVVALpYDYMPM (B22)  |



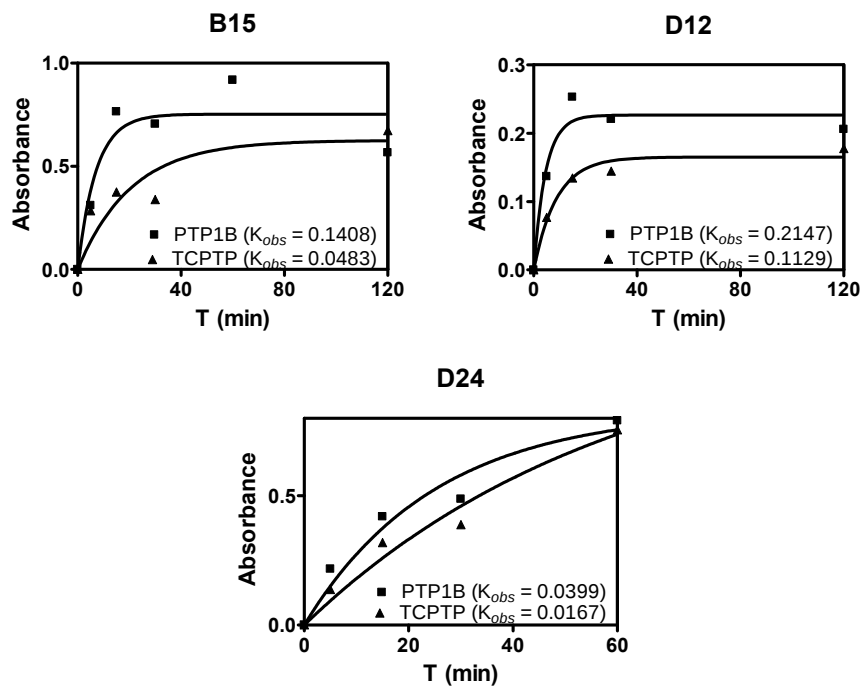
**Figure S9.** Venn diagram comparing the top-20 sequences between the single-point data and  $K_D$  results.

### 3.3. Dual-color screening experiments.

Dual-color screening with PTP1B/TCPTP, full-length SHP1/SHP2 and  $\Delta$ SHP1/ $\Delta$ SHP2 were carried out, and the complete results were summarized in the supplementary excel file “SI2\_Sun.xls”. Selected peptides were further characterized by (1) determining  $k_{obs}$  using microplate-based enzymatic PTP assay with the corresponding wildtype PTPs, or/and (2) comparing the  $K_D$  ratio obtained from the microarray-based  $K_D$  experiments. Results of particular interest are summarized in Tables S4-S6 and Figures S10 & S11.

**Table S4.** The  $k_{obs}$  of PTP1B-selective peptides against PTP1B and TCPTP. The peptides were identified from the dual-color PTP1B/TCPTP ratiometric screening results (e.g. Figure 4 in the maintext).

| ID  | Peptide Sequence      | Enzyme targeted/protein source | $k_{obs}$<br>(obtained from enzymatic assay) |       |
|-----|-----------------------|--------------------------------|----------------------------------------------|-------|
|     |                       |                                | PTP1B                                        | TCPTP |
| B15 | IESDI <b>pY</b> AEIPD | Type 12                        | 0.14                                         | 0.048 |
| D12 | SSTVQ <b>pY</b> STVVH | SHP2                           | 0.215                                        | 0.11  |
| D24 | YFMTE <b>pY</b> VATRW | Mitogen kinase                 | 0.040                                        | 0.017 |



**Figure S10.** Microplate-based enzymatic PTP assay to determine the  $k_{obs}$  of the four selected peptides against PTP1B and TCPTP.

**Table S5.**  $K_D$  of selected peptides identified from the dual-color SHP1/SHP2 ratiometric screening results (e.g. Figure 4 in the maintext).  $K_D$  results were extracted from the microarray-based  $K_D$  experiments. Shaded are entries that overlap with those in Table S6.

| ID  | Peptide sequence | Enzyme targeted | Protein source   | $K_D$ ( $\mu$ M) |                 | SHP1/SHP2 ratio (obtained from dual-color experiments) |
|-----|------------------|-----------------|------------------|------------------|-----------------|--------------------------------------------------------|
|     |                  |                 |                  | SHP1             | SHP2            |                                                        |
| A18 | EGVATpYAAAVL     | SHP1            | CATENIN (654)    | 0.96             | 1.1             | 9.4                                                    |
| C19 | PSFSEpYASVQV     | SHP2            | SIRP (496)       | 0.93             | 0.62            | 4.3                                                    |
| E19 | AELEFpYMDYEA     | PTP1B           | Alanine scanning | 0.41             | NA <sup>a</sup> | 17.7                                                   |
| E21 | GPQDIpYDVPPV     |                 | P130cas          | 1.1              | NA              | 12.7                                                   |
| B06 | GNNYVpY IDPTQ    | SHP1            | KIT (570)        | NA               | NA              | 5.3                                                    |
| B15 | IESDIpYAEIPD     | Type12          | FAK2             | 0.2              | NA              | 27                                                     |

<sup>a</sup>NA indicates that either  $K_D$  can not be accurately determined due to (1) weak binding from fluorescence signals, and/or (2) too much nonspecific binding preventing the saturation binding curve to be fitted properly.

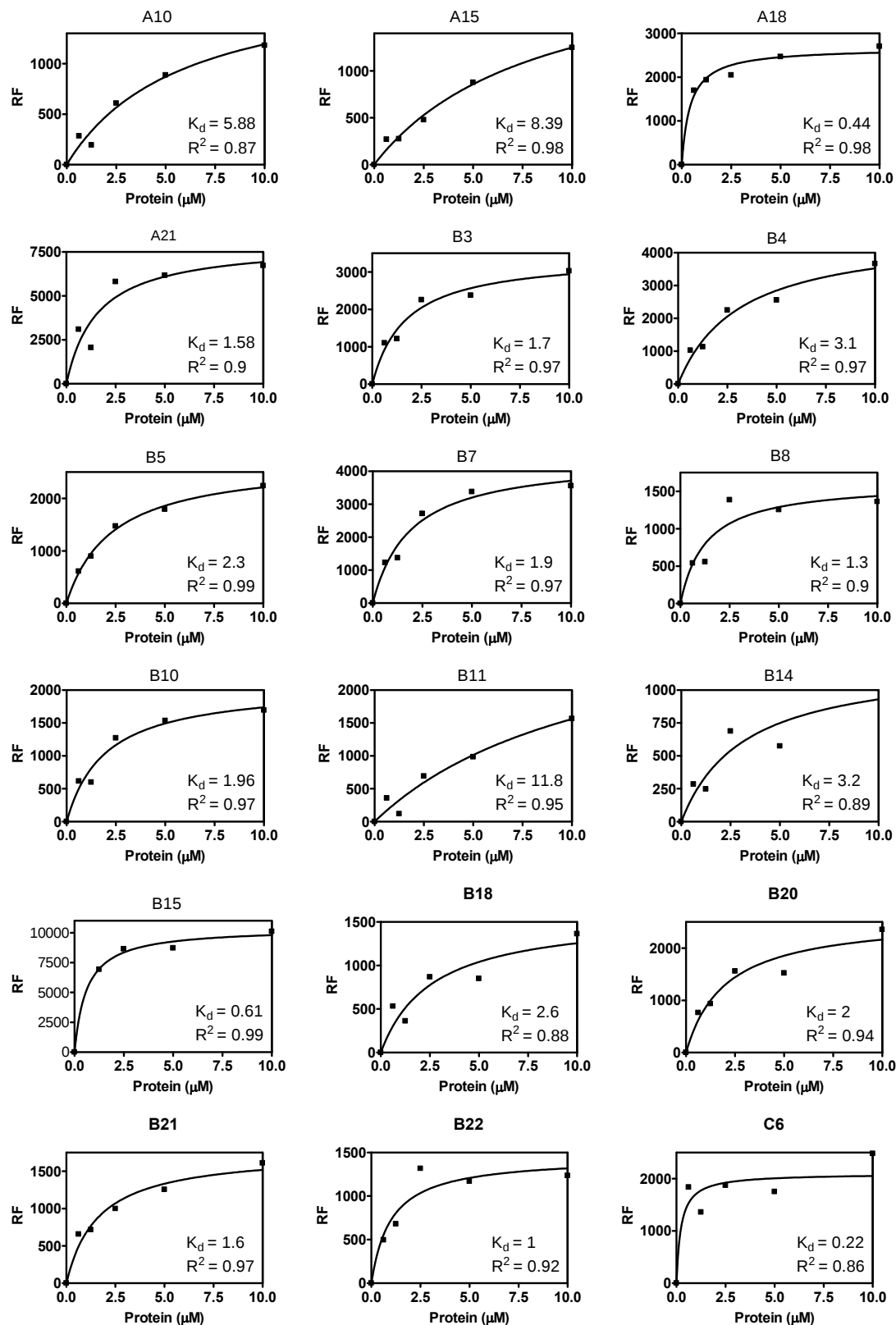
**Table S6.**  $K_D$  of selected peptides identified from the dual-color  $\Delta$ SHP1/ $\Delta$ SHP2 ratiometric screening results (e.g. Figure 4 in the maintext).  $K_D$  results were extracted from the microarray-based  $K_D$  experiments. Shaded are entries that overlap with those in Table S6.

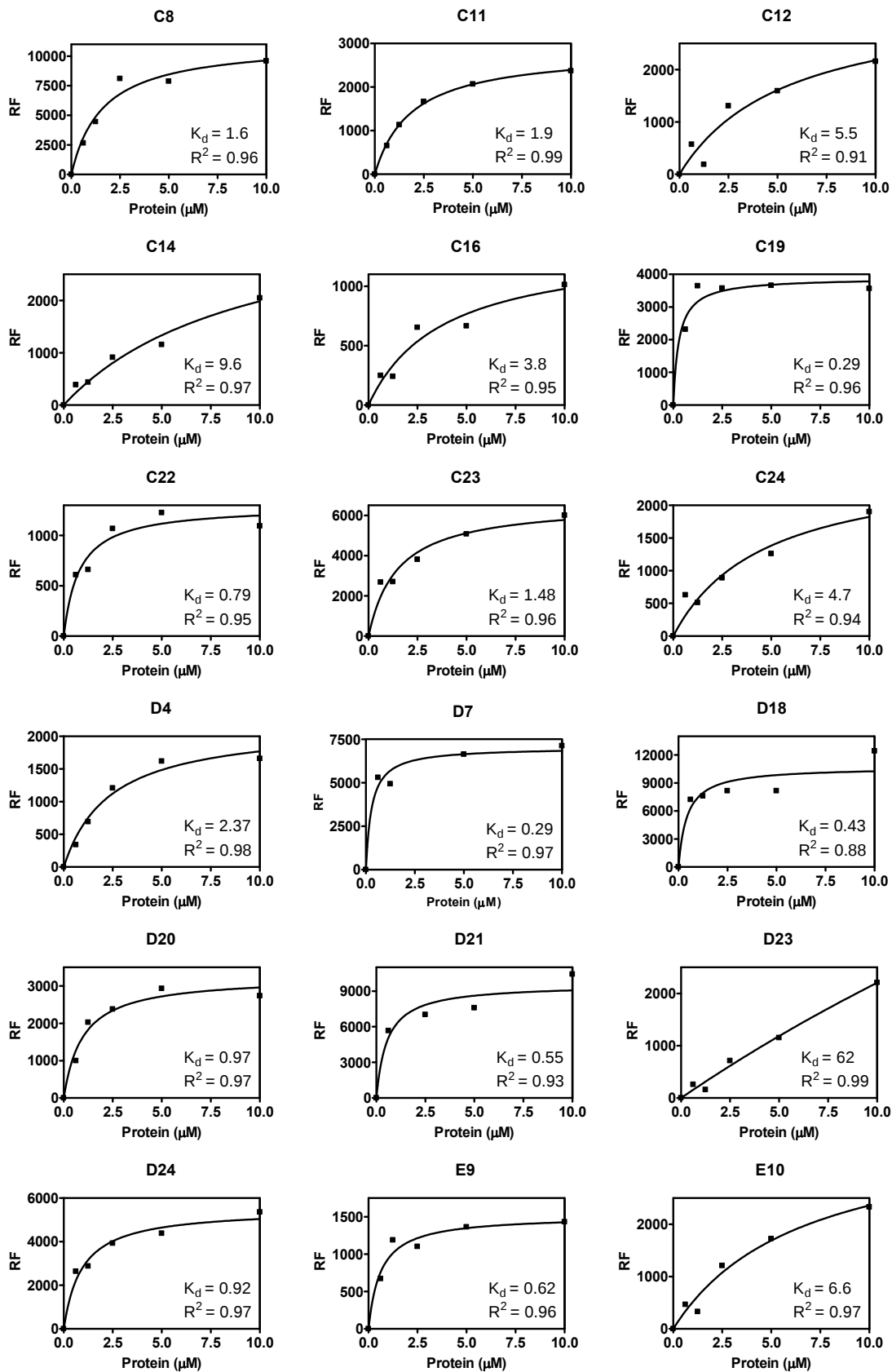
| ID  | Peptide Sequence | Enzyme targeted | Protein Source   | $K_D$ ( $\mu$ M) |                 | $\Delta$ SHP1/ $\Delta$ SHP2 ratio (obtained from dual-color experiments) |
|-----|------------------|-----------------|------------------|------------------|-----------------|---------------------------------------------------------------------------|
|     |                  |                 |                  | SHP1             | SHP2            |                                                                           |
| A18 | EGVATpYAAAVL     | SHP1            | CATENIN          | 0.96             | 1.1             | 7.6                                                                       |
| C19 | PSFSEpYASVQ<br>V | SHP2            | SIRP             | 0.93             | 0.62            | 4.3                                                                       |
| E19 | AELEFpYMDYE<br>A | PTP1B           | Alanine scanning | 0.41             | NA <sup>a</sup> | 6.6                                                                       |
| B06 | GNNYVpY<br>IDPTQ | SHP1            | KIT              | NA               | NA              | 4                                                                         |
| B15 | IESDIpYAEIPD     | Type12          | FAK2             | 0.2              | NA              | 4.5                                                                       |

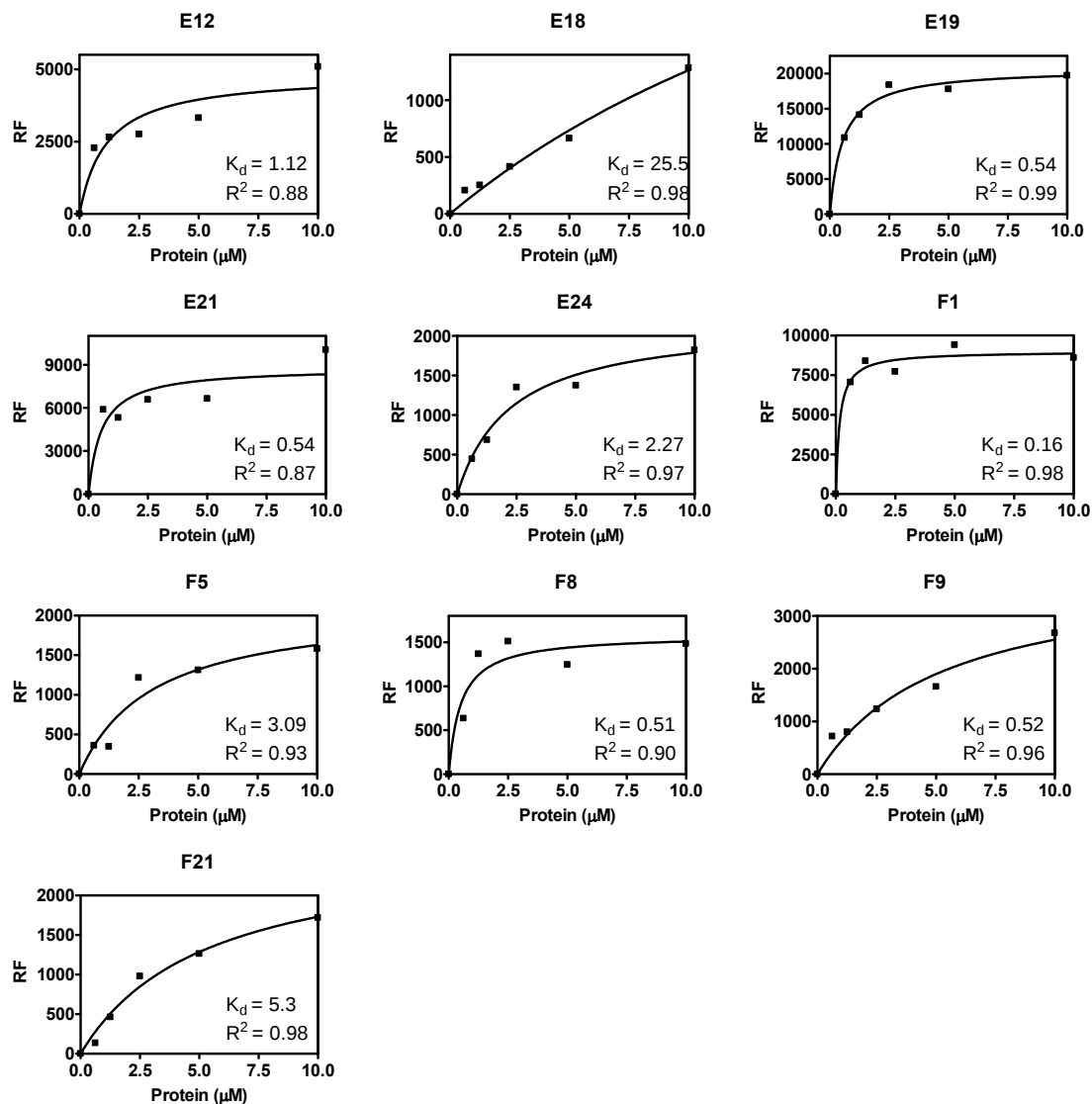
<sup>a</sup>NA indicates that either  $K_D$  can not be accurately determined due to (1) weak binding from fluorescence signals, and/or (2) too much nonspecific binding preventing the saturation binding curve to be fitted properly.

### 3.4. Fitted $K_D$ curves obtained from microarray $K_D$ experiments.

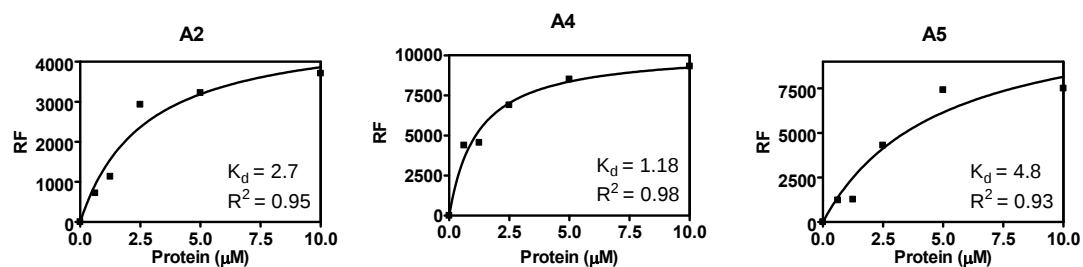
(a) PTP1B

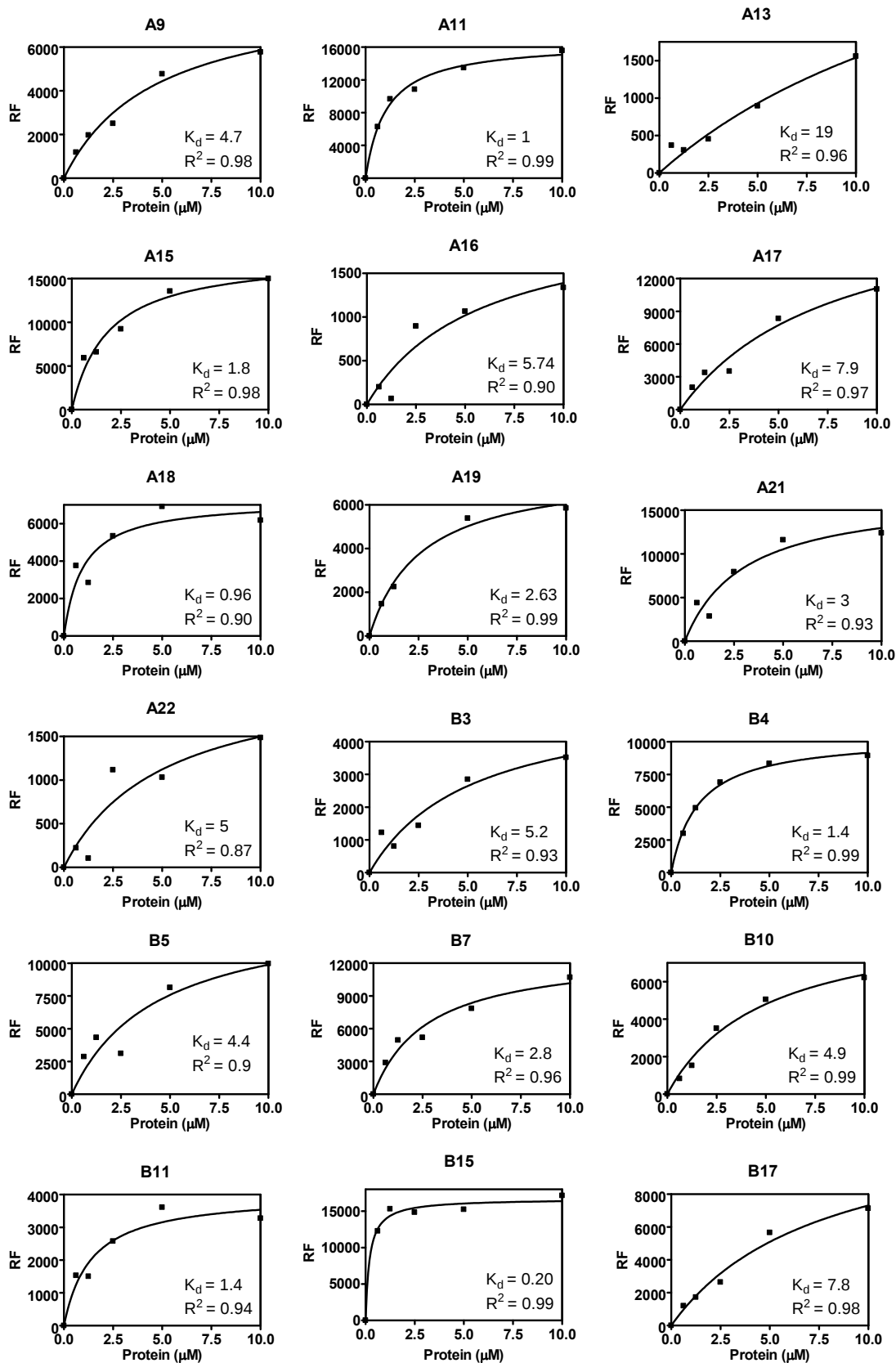


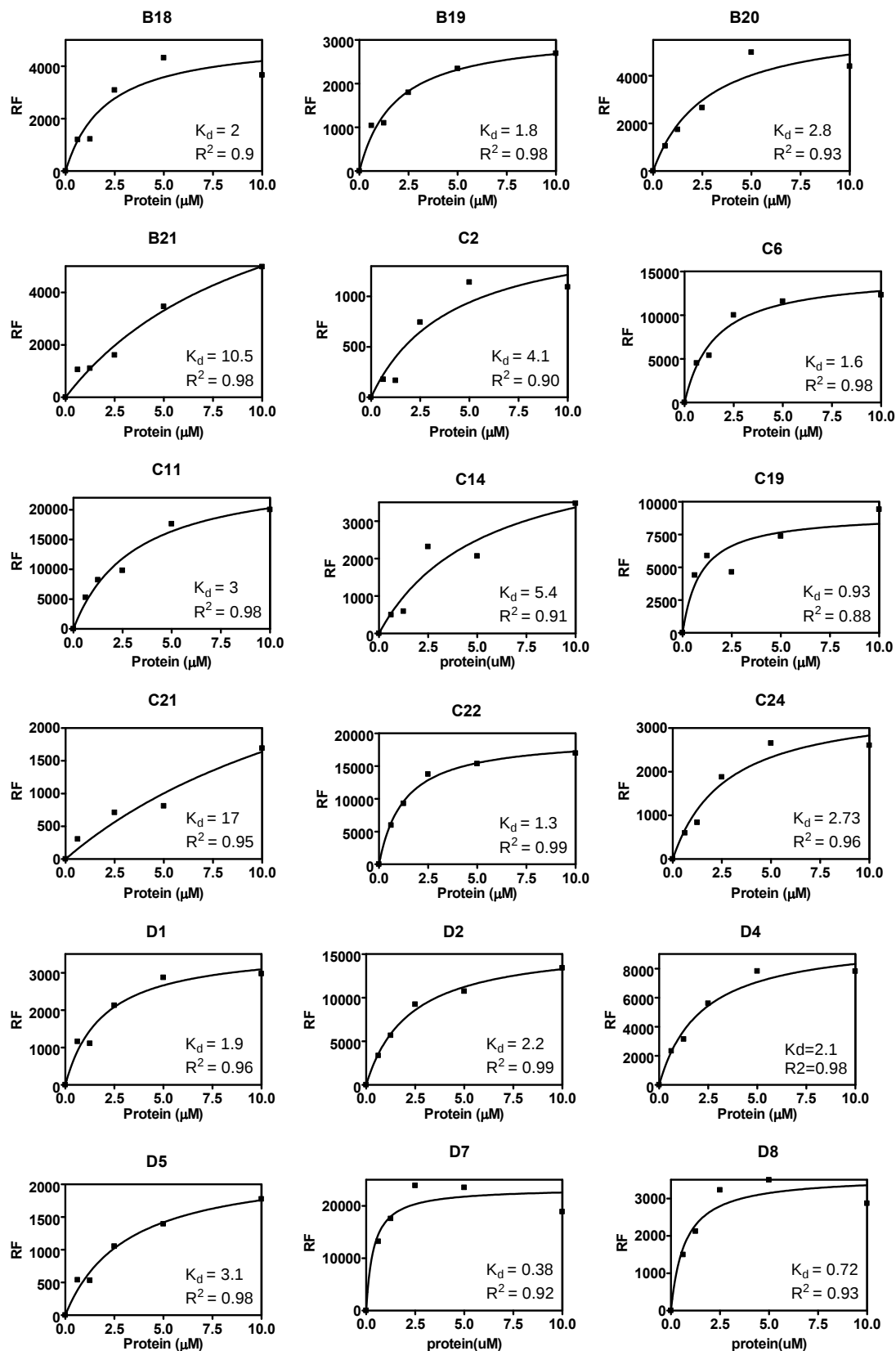


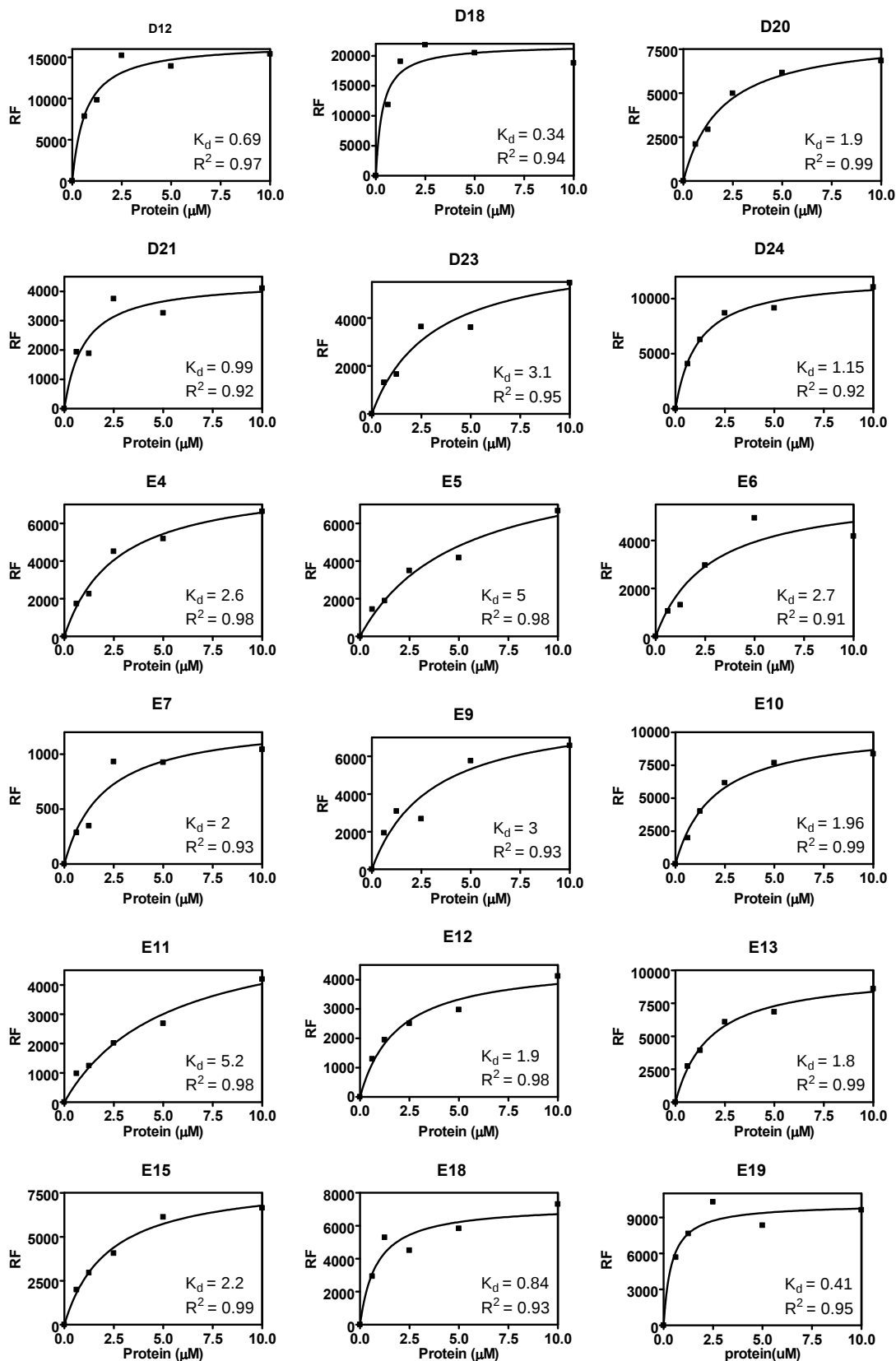


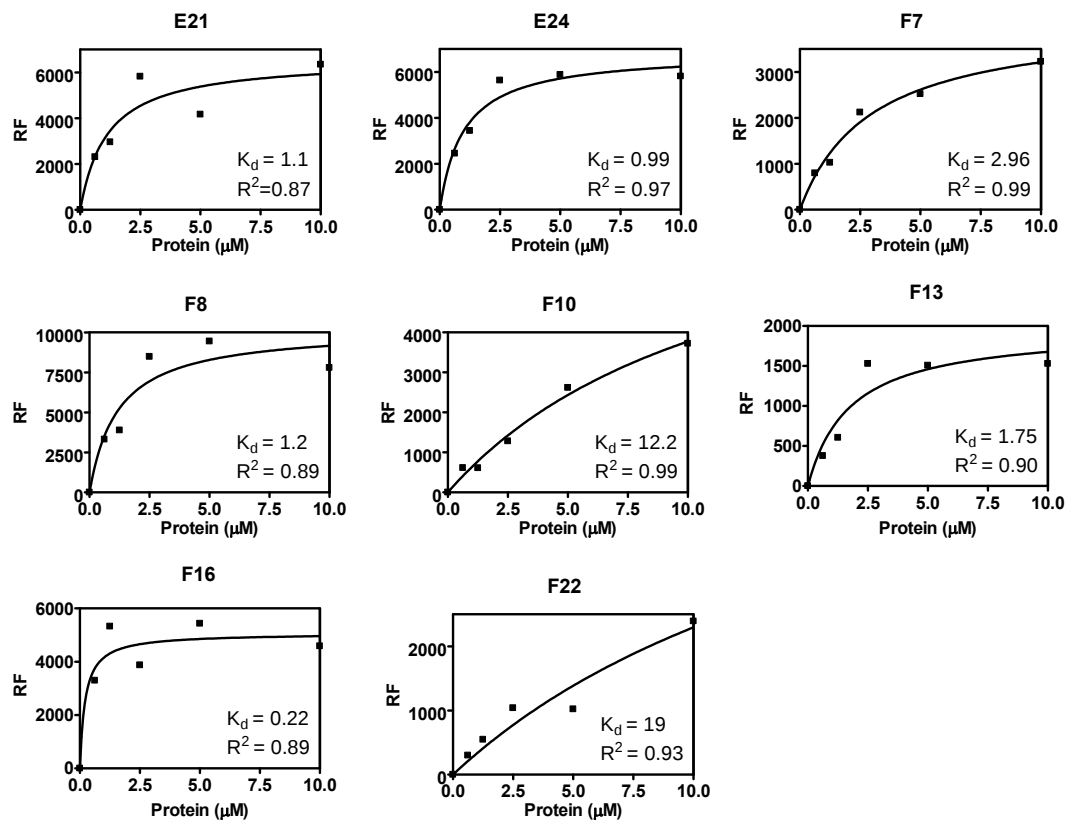
(b) Full-length SHP1



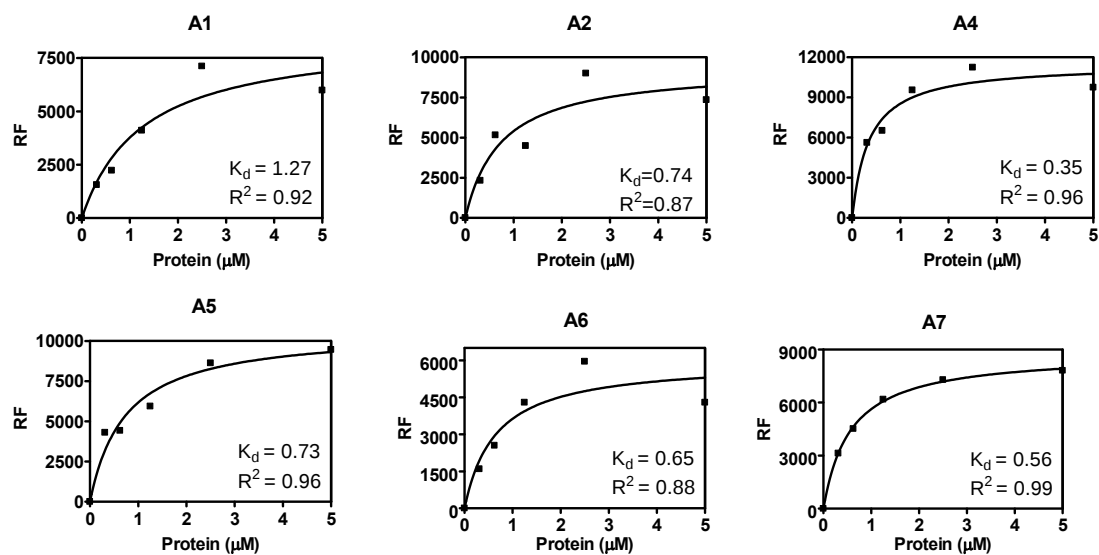


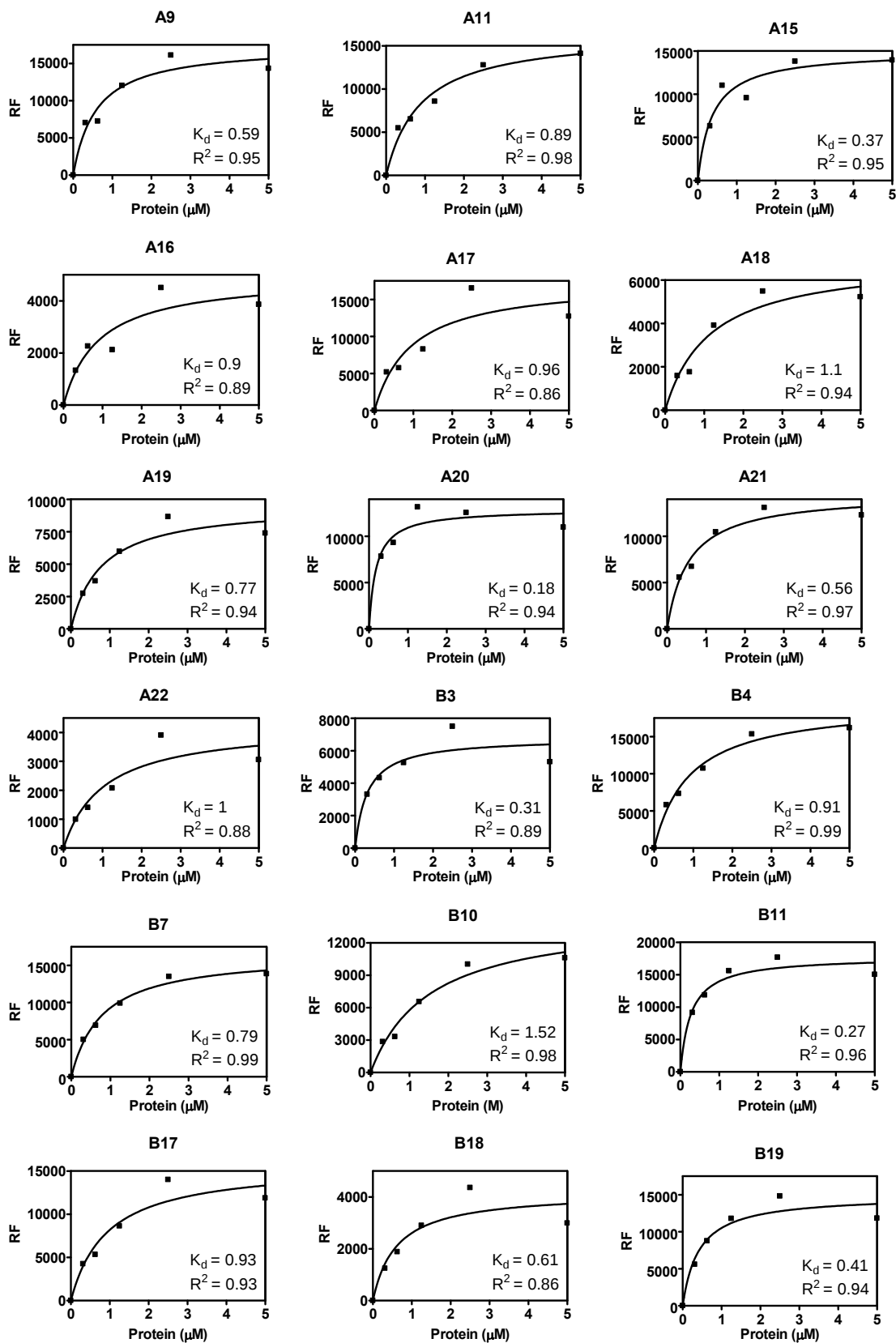


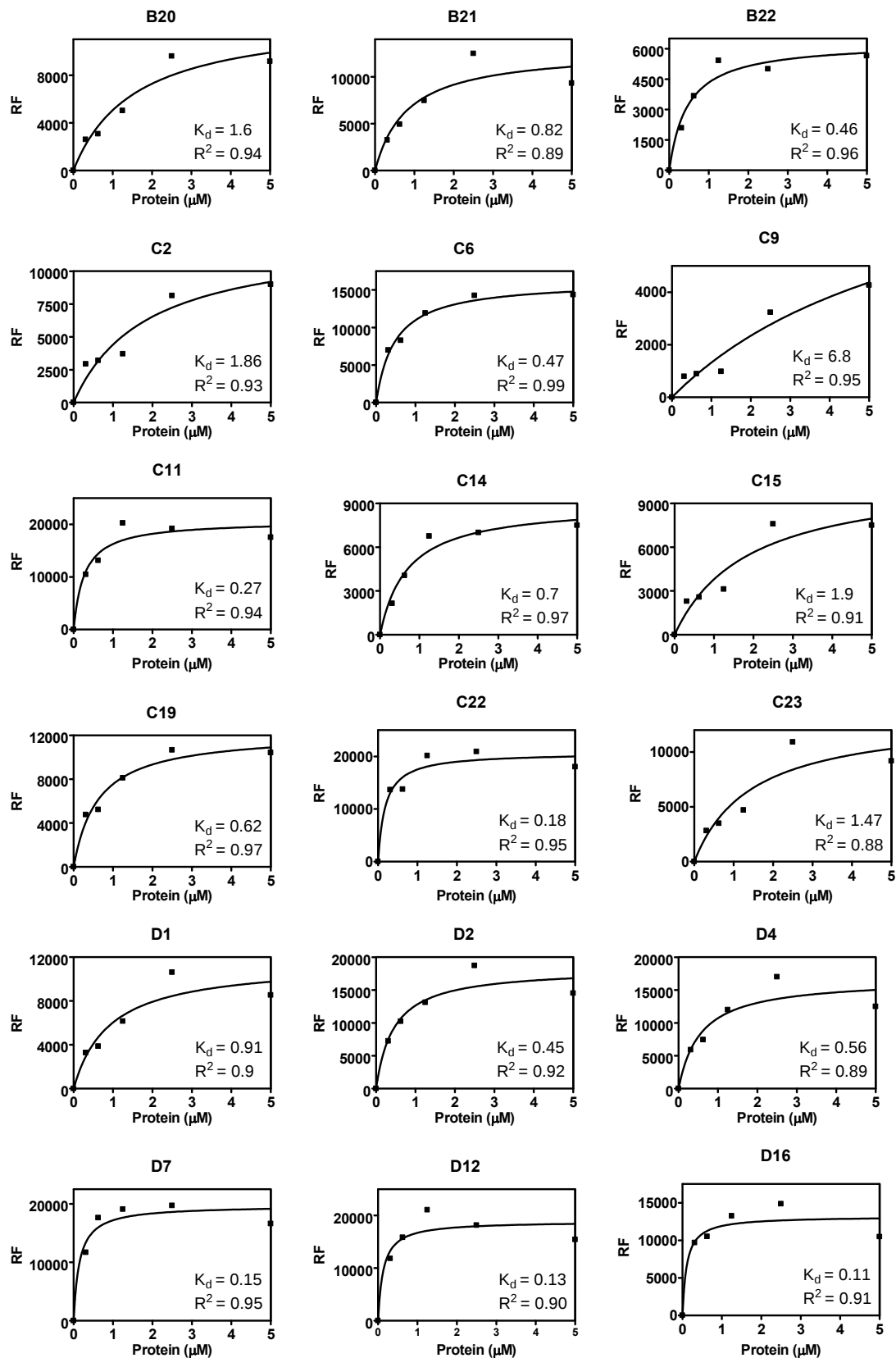


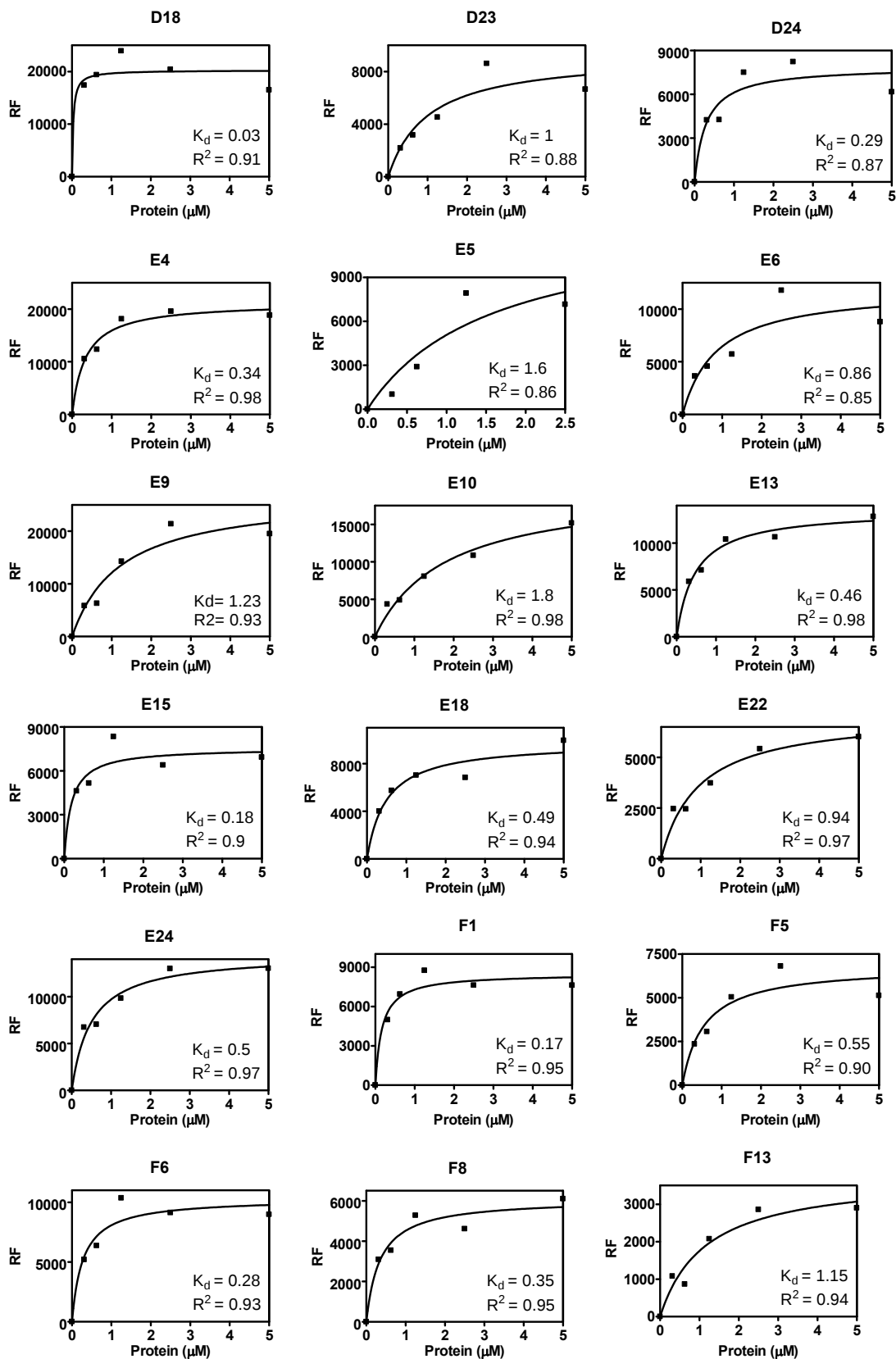


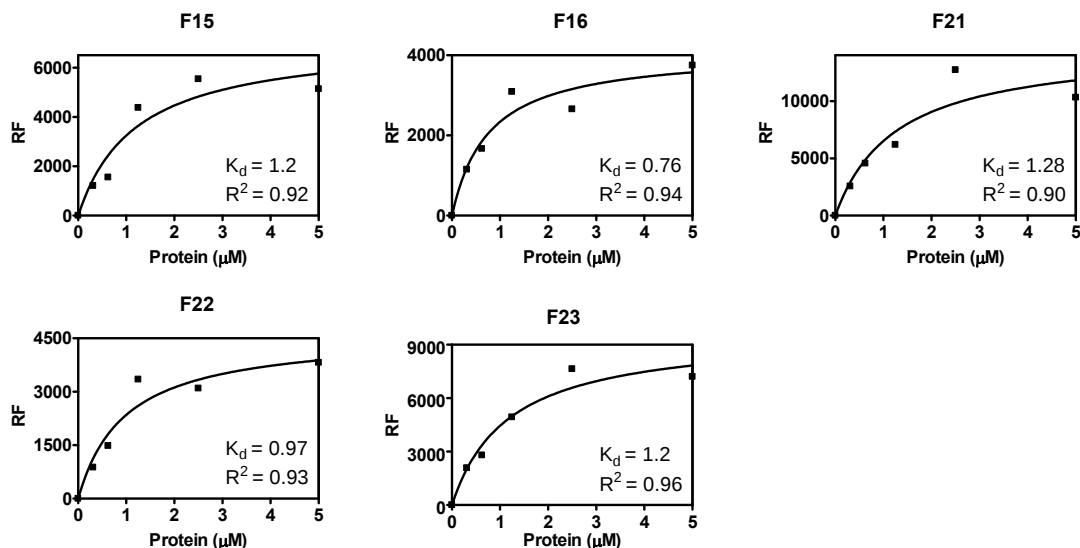
(c) Full-length SHP2



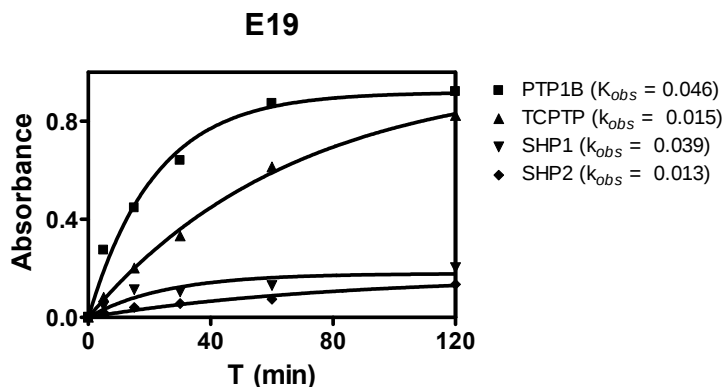








**Figure S11.** Fitted  $K_D$  curves obtained from microarray  $K_D$  experiments. Results were summarized in the supplementary excel file “SI2\_Sun.xls”. Selected data were represented in Tables S5 & S6.  
(a) PTP1B, (b) SHP1, (c) SHP2.



**Figure S12.** Microplate-based enzymatic PTP assay to determine the  $k_{obs}$  of E19 peptide against the four PTPs (PTP1B, TCPTP, SHP1 and SHP2).

#### 4. References

- [1] (a) Lu, C.H.S.; Sun, H.; Bakar, F.B.A.; Uttamchandani, M.; Zhou, W.; Liou, Y.-C.; Yao, S.Q., *Angew. Chem. Int. Ed.*, **2008**, 47, 7438-7441. (b) Sun, H.; Lu, C.H.S.; Uttamchandani, M.; Xia, Y.; Liou, Y.-C.; Yao, S.Q., *Angew. Chem. Intl. Ed.*, **2008**, 47, 1698-1702. (c) Uttamchandani, M.; Lee, W.L.; Wang, J.; Yao, S.Q., *J. Am. Chem. Soc.*, **2007**, 129, 13110-13117.
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Single-point data

| NO. | ID  | Sequence      | Absolute Fluorescence Intensity |                      |                     |                     |                       |
|-----|-----|---------------|---------------------------------|----------------------|---------------------|---------------------|-----------------------|
|     |     |               | PTP1B<br>(1 $\mu$ M)            | TCPTP<br>(1 $\mu$ M) | SHP1<br>(1 $\mu$ M) | SHP2<br>(1 $\mu$ M) | LMWPTP<br>(1 $\mu$ M) |
| 1   | A01 | AEKPFpYVNVEF  | 249                             | 193                  | 222                 | 3462                | 126                   |
| 2   | A02 | AEMTGpYVVTRW  | 1171                            | 864                  | 1136                | 5527                | 5111                  |
| 3   | A03 | AENAEpYLRVAP  | 0                               | 0                    | 445                 | 2352                | 0                     |
| 4   | A04 | DEELHpYASLNF  | 435                             | 169                  | 4557                | 10457               | 1027                  |
| 5   | A05 | DEGIHpYSELIQ  | 428                             | 917                  | 1271                | 9968                | 219                   |
| 6   | A06 | DEKVDpYVQVDK  | 51                              | 0                    | 58                  | 3009                | 612                   |
| 7   | A07 | DERVDpYVVVDQ  | 0                               | 22                   | 472                 | 4016                | 806                   |
| 8   | A08 | DESVDpYVPM LD | 587                             | 66                   | 29                  | 54                  | 352                   |
| 9   | A09 | DKQVEpYLDL DL | 56                              | 19                   | 1974                | 8898                | 437                   |
| 10  | A10 | DSGGFpYITSRT  | 1181                            | 112                  | 178                 | 1233                | 1289                  |
| 11  | A11 | DTETVpYSEVRK  | 588                             | 118                  | 9680                | 9670                | 2350                  |
| 12  | A12 | EANSHpYGHND D | 0                               | 0                    | 0                   | 48                  | 834                   |
| 13  | A13 | EDEDYpYKASVT  | 321                             | 140                  | 305                 | 1095                | 604                   |
| 14  | A14 | EDGGVpYSSSGL  | 380                             | 282                  | 0                   | 859                 | 1956                  |
| 15  | A15 | EDGISpYTTLRF  | 1248                            | 586                  | 6588                | 12343               | 1797                  |
| 16  | A16 | EDSTYpYKASKG  | 468                             | 68                   | 66                  | 818                 | 1593                  |
| 17  | A17 | EDTLTpYADLDM  | 251                             | 355                  | 3399                | 12782               | 229                   |
| 18  | A18 | EGVATpYAAAVL  | 2705                            | 3936                 | 2849                | 3002                | 375                   |
| 19  | A19 | EGDNDpYIILP   | 683                             | 378                  | 2252                | 5374                | 378                   |
| 20  | A20 | ENGLNpYIDL DL | 944                             | 997                  | 1392                | 12973               | 403                   |
| 21  | A21 | EPNVSpYICSR Y | 6722                            | 6488                 | 2853                | 10213               | 5336                  |
| 22  | A22 | ESDGSpYQKPS Y | 781                             | 350                  | 105                 | 972                 | 2942                  |
| 23  | A23 | ESDGGpYMDMS K | 585                             | 0                    | 304                 | 1863                | 2278                  |
| 24  | A24 | ETDKEpY YTVKD | 0                               | 0                    | 0                   | 154                 | 62                    |
| 25  | B01 | FEEDDpYESPND  | 0                               | 0                    | 0                   | 0                   | 0                     |
| 26  | B02 | FGMTRpYVLDDE  | 43                              | 291                  | 135                 | 814                 | 165                   |
| 27  | B03 | FLFNMpYLTRER  | 3025                            | 1875                 | 806                 | 3317                | 5257                  |
| 28  | B04 | FMMTPpYVVTRY  | 3664                            | 2531                 | 4939                | 7381                | 9586                  |
| 29  | B05 | GFLTEpYVATRW  | 2238                            | 837                  | 4328                | 3926                | 3147                  |
| 30  | B06 | GNNYVpYIDPTQ  | 1538                            | 524                  | 1758                | 1433                | 325                   |
| 31  | B07 | GSAAPpYLKTKF  | 3557                            | 950                  | 4957                | 4457                | 12409                 |
| 32  | B08 | GWMEDpYDYVHL  | 1362                            | 876                  | 1044                | 719                 | 663                   |
| 33  | B09 | GWMVHpYTSKDT  | 90                              | 0                    | 178                 | 1406                | 1045                  |
| 34  | B10 | HNSALpYSQVQK  | 1695                            | 707                  | 1528                | 3328                | 4753                  |
| 35  | B11 | HRQLNpYIQVDL  | 1565                            | 574                  | 1501                | 10960               | 2762                  |
| 36  | B12 | IEDNEpYTAREG  | 834                             | 0                    | 0                   | 1339                | 192                   |
| 37  | B13 | IEDEDpYYKASV  | 524                             | 111                  | 1077                | 1600                | 1946                  |
| 38  | B14 | IEDNEpYTARQG  | 1003                            | 0                    | 119                 | 1186                | 829                   |
| 39  | B15 | IESDlpYAEIPD  | 10095                           | 12914                | 15308               | 11860               | 288                   |
| 40  | B16 | IESSNpYMAPYD  | 1051                            | 793                  | 600                 | 2657                | 0                     |
| 41  | B17 | IRYHRpYHGRSA  | 1559                            | 791                  | 1723                | 3198                | 10879                 |
| 42  | B18 | IYETDpYYRKGG  | 1364                            | 833                  | 1220                | 1155                | 3528                  |
| 43  | B19 | KAVDGpYVKPQI  | 767                             | 271                  | 1099                | 5420                | 4579                  |
| 44  | B20 | KDRMSpYHVRS H | 2355                            | 558                  | 1741                | 2722                | 6890                  |
| 45  | B21 | KKRCPpYTKHQT  | 1609                            | 683                  | 1105                | 3174                | 8123                  |
| 46  | B22 | KVVALpYDYMPM  | 1235                            | 852                  | 1738                | 3630                | 4748                  |
| 47  | B23 | LISSDpYELLSD  | 39                              | 158                  | 285                 | 1196                | 0                     |
| 48  | B24 | LNSDGpYTPEPA  | 0                               | 0                    | 0                   | 268                 | 0                     |

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|    |     |                |       |       |       |       |       |
|----|-----|----------------|-------|-------|-------|-------|-------|
| 49 | C01 | LNSKGpYTKSID   | 0     | 0     | 128   | 830   | 1037  |
| 50 | C02 | LPPEGpYVVVVVK  | 1315  | 243   | 167   | 3076  | 11155 |
| 51 | C03 | MDTSVpYESPYS   | 161   | 0     | 51    | 661   | 995   |
| 52 | C04 | MKDEEpYEQMVK   | 596   | 0     | 37    | 911   | 2910  |
| 53 | C05 | MTGDTpYTAHAG   | 491   | 0     | 137   | 916   | 1165  |
| 54 | C06 | NNHTEpYASIQT   | 2480  | 2102  | 5397  | 8582  | 1560  |
| 55 | C07 | NQSSGpYRYGTD   | 0     | 0     | 28    | 1286  | 1148  |
| 56 | C08 | NSDVQpYTEVQV   | 9583  | 6459  | 1382  | 7188  | 2166  |
| 57 | C09 | NSKRDpYTGCT    | 602   | 0     | 225   | 730   | 2425  |
| 58 | C10 | NVVPLpYDLLLE   | 688   | 200   | 0     | 576   | 445   |
| 59 | C11 | PEGLNpYACLTH   | 2371  | 2290  | 9095  | 17829 | 2233  |
| 60 | C12 | PEGHEpYpYRVRE  | 2158  | 1810  | 0     | 956   | 623   |
| 61 | C13 | PEQDEpYDIPRH   | 153   | 0     | 704   | 1323  | 336   |
| 62 | C14 | PGSLEpYLCLPA   | 2047  | 1697  | 589   | 5226  | 418   |
| 63 | C15 | PKGTGpYIKTEL   | 1006  | 86    | 709   | 3183  | 3217  |
| 64 | C16 | PLDKDpYpYVVRE  | 1013  | 241   | 0     | 932   | 571   |
| 65 | C17 | PPDHQpYYNDFP   | 125   | 50    | 123   | 751   | 48    |
| 66 | C18 | PQDKEpYKVKKE   | 484   | 0     | 0     | 444   | 1081  |
| 67 | C19 | PSFSEpYASVQV   | 3564  | 3853  | 5876  | 5283  | 2771  |
| 68 | C20 | PSTRDpYEIQRE   | 0     | 0     | 0     | 88    | 0     |
| 69 | C21 | PQDKEpYpYKVKKE | 1229  | 1909  | 396   | 1963  | 1702  |
| 70 | C22 | QGPVI pYAQLDH  | 1095  | 676   | 9279  | 19158 | 2309  |
| 71 | C23 | QKQIpYIVMEL    | 6003  | 9871  | 1808  | 1234  | 1769  |
| 72 | C24 | QQQEVpYGMMPR   | 1902  | 538   | 837   | 1665  | 4117  |
| 73 | D01 | RNEGVpYTAIAV   | 1085  | 2040  | 1106  | 1188  | 1413  |
| 74 | D02 | REGLNpYMLAT    | 2574  | 2088  | 5667  | 11520 | 3622  |
| 75 | D03 | SAEPQpYQPGDQ   | 0     | 48    | 51    | 66    | 164   |
| 76 | D04 | SDDVRpYVNAFK   | 1660  | 683   | 3147  | 6727  | 3237  |
| 77 | D05 | SDGHEpYIYVDP   | 42    | 0     | 531   | 2295  | 1144  |
| 78 | D06 | SEEPi pYIVTEY  | 0     | 0     | 310   | 750   | 0     |
| 79 | D07 | SESVVpYADIRK   | 7119  | 6577  | 17579 | 19127 | 5101  |
| 80 | D08 | SETDDpYAEIID   | 1748  | 2140  | 2122  | 4859  | 0     |
| 81 | D09 | SPPALpYAEPLD   | 606   | 122   | 0     | 1028  | 822   |
| 82 | D10 | SSNPEpYLSASD   | 227   | 0     | 0     | 424   | 0     |
| 83 | D11 | SSSSEpYGSVSP   | 81    | 0     | 382   | 1574  | 1000  |
| 84 | D12 | SSTVQpYSTVVH   | 6184  | 7606  | 9834  | 15367 | 5234  |
| 85 | D13 | STEPQpYQPGEN   | 446   | 0     | 0     | 0     | 526   |
| 86 | D14 | TDKEYpYTVKDD   | 358   | 0     | 0     | 757   | 0     |
| 87 | D15 | TGGSVpYTEDND   | 482   | 0     | 0     | 513   | 482   |
| 88 | D16 | TNDITpYADLNL   | 484   | 75    | 4384  | 10029 | 704   |
| 89 | D17 | TSKLI pYDFIED  | 0     | 0     | 346   | 1613  | 397   |
| 90 | D18 | TSSVLpYTAVQP   | 12415 | 10065 | 19048 | 20727 | 1270  |
| 91 | D19 | VDADEpYLIPQQ   | 250   | 0     | 531   | 1875  | 0     |
| 92 | D20 | VDTPHpYPRWIT   | 2735  | 883   | 2926  | 4104  | 4564  |
| 93 | D21 | VNTTLpYEKFTY   | 10414 | 13706 | 1884  | 7611  | 11381 |
| 94 | D22 | VYESPpYSDPEE   | 0     | 0     | 658   | 0     | 284   |
| 95 | D23 | YETDYpYRKGGK   | 2208  | 314   | 1651  | 2423  | 6362  |
| 96 | D24 | YFMTEpYVATRW   | 5359  | 3723  | 6285  | 3795  | 4279  |
| 97 | E01 | AEGSa pYEEVPT  | 0     | 0     | 4     | 1744  | 0     |
| 98 | E02 | AENPEpYLSEFS   | 698   | 458   | 520   | 622   | 2040  |
| 99 | E03 | FDNLYpYWDQDP   | 125   | 1319  | 730   | 29    | 504   |

Single-point data

|     |     |              |       |       |       |      |       |
|-----|-----|--------------|-------|-------|-------|------|-------|
| 100 | E04 | AFGTVpYKGIWI | 3281  | 1796  | 2258  | 7640 | 13900 |
| 101 | E05 | AFQFSpYTAVFG | 1286  | 438   | 1888  | 4469 | 1323  |
| 102 | E06 | AKIQDpYHILTR | 1535  | 453   | 1314  | 3274 | 4850  |
| 103 | E07 | APAEMpYDIMKT | 783   | 1162  | 347   | 896  | 1545  |
| 104 | E08 | ASEQSpYEEMRA | 696   | 0     | 0     | 436  | 75    |
| 105 | E09 | ATVGHpYTAVQN | 1431  | 2891  | 3089  | 5459 | 893   |
| 106 | E10 | AYRQLpYLNPKG | 2329  | 945   | 4011  | 4092 | 5038  |
| 107 | E11 | CPEKVpYELMRA | 2408  | 943   | 1241  | 2336 | 555   |
| 108 | E12 | DINSLpYDVSRM | 5096  | 5805  | 1946  | 1484 | 3554  |
| 109 | E13 | DRFIQpYANPAF | 1090  | 1100  | 3926  | 8788 | 2062  |
| 110 | E14 | DVSRMpYVDPSE | 5048  | 4868  | 0     | 1847 | 1040  |
| 111 | E15 | EDIKSpYYTVRQ | 1113  | 1007  | 2951  | 4304 | 4370  |
| 112 | E16 | EEIRFpYQLGEE | 174   | 0     | 0     | 669  | 610   |
| 113 | E17 | EKIQDpYEKMPE | 0     | 0     | 0     | 61   | 0     |
| 114 | E18 | ELGYEpYMDVGS | 1285  | 1034  | 5289  | 3714 | 3386  |
| 115 | E19 | AELEFpYMDYEA | 19714 | 17603 | 7643  | 7998 | 2470  |
| 116 | E20 | FGAKPpYDGIPA | 1186  | 18    | 303   | 1139 | 2105  |
| 117 | E21 | GPQDlpYDVPPV | 10036 | 8562  | 2960  | 3041 | 1288  |
| 118 | E22 | GQSESpYGNITY | 440   | 340   | 773   | 1126 | 2114  |
| 119 | E23 | GRETlpYPNASL | 1180  | 623   | 190   | 612  | 1298  |
| 120 | E24 | IGEGTpYGTVFK | 1820  | 561   | 3439  | 4191 | 5041  |
| 121 | F01 | IYIHRpYENVSI | 8592  | 12658 | 2995  | 6891 | 2449  |
| 122 | F02 | KAEDEpYVNEPL | 662   | 0     | 0     | 1842 | 0     |
| 123 | F03 | KPKQEpYLNPE  | 0     | 0     | 231   | 1089 | 0     |
| 124 | F04 | LARDMpYDKEYY | 903   | 0     | 438   | 1546 | 339   |
| 125 | F05 | LDSTFpYRSLLE | 1582  | 1312  | 882   | 3838 | 117   |
| 126 | F06 | LGQRlpYQYIQS | 3196  | 3509  | 2115  | 5032 | 3243  |
| 127 | F07 | LLANApYIYVVQ | 510   | 346   | 1025  | 1785 | 988   |
| 128 | F08 | LMGHEpYMEMKN | 1484  | 470   | 3891  | 3457 | 2220  |
| 129 | F09 | LNKQSpYKCRQC | 2677  | 1274  | 12417 | 6322 | 1387  |
| 130 | F10 | NKPTVpYGVSPN | 486   | 0     | 608   | 1945 | 2149  |
| 131 | F11 | PPDHQpYYNDFP | 599   | 478   | 958   | 1695 | 0     |
| 132 | F12 | PEDTFpYFDPEF | 538   | 503   | 216   | 1881 | 267   |
| 133 | F13 | PEPGPpYAQPSV | 0     | 0     | 605   | 987  | 362   |
| 134 | F14 | RHDSGpYEVHHQ | 738   | 0     | 215   | 689  | 1260  |
| 135 | F15 | RNPGFpYVEANP | 611   | 129   | 407   | 2449 | 0     |
| 136 | F16 | SADHLpYVNVSE | 3330  | 1878  | 5325  | 1486 | 337   |
| 137 | F17 | SSDPTpYTSSLG | 144   | 0     | 0     | 1277 | 0     |
| 138 | F18 | EEEPVpYEAPE  | 1424  | 0     | 38    | 241  | 398   |
| 139 | F19 | STKYFpYKQNGR | 860   | 503   | 3461  | 1018 | 33    |
| 140 | F20 | TAEPDpYGALYE | 1080  | 361   | 40    | 1755 | 0     |
| 141 | F21 | VCAERpYSQEVF | 1719  | 1867  | 4141  | 4766 | 139   |
| 142 | F22 | VDSSLpYNLPRS | 360   | 104   | 547   | 1770 | 762   |
| 143 | F23 | VSSTHpYLLPE  | 0     | 0     | 2234  | 2733 | 80    |
| 144 | F24 | VVIALpYDYQTN | 0     | 0     | 377   | 351  | 0     |

PTP1B Kd

| NO. | ID  | Sequence      | Fluorescence  |              |             |           |            | PTP1B<br>Kd/ $\mu$ M | R <sup>2</sup> |
|-----|-----|---------------|---------------|--------------|-------------|-----------|------------|----------------------|----------------|
|     |     |               | 0.625 $\mu$ M | 1.25 $\mu$ M | 2.5 $\mu$ M | 5 $\mu$ M | 10 $\mu$ M |                      |                |
| 1   | A10 | DSGGFpYITSRT  | 283           | 195          | 609         | 888       | 1181       | 5.88                 | 0.97           |
| 2   | A15 | EDGISpYTTLRF  | 269           | 275          | 477         | 877       | 1248       | 8.39                 | 0.98           |
| 3   | A18 | EGVATpYAAAVL  | 1697          | 1937         | 2048        | 2468      | 2705       | 0.43                 | 0.98           |
| 4   | A21 | EPNVSpYICSR   | 3096          | 2055         | 5804        | 6168      | 6722       | 1.58                 | 0.9            |
| 5   | B03 | FLFNMpYLTRER  | 1098          | 1211         | 2253        | 2372      | 3025       | 1.7                  | 0.97           |
| 6   | B04 | FMMTPpYVVTRY  | 1024          | 1133         | 2249        | 2557      | 3664       | 3.1                  | 0.97           |
| 7   | B05 | GFLTEpYVATRW  | 612           | 896          | 1472        | 1792      | 2238       | 2.3                  | 0.99           |
| 8   | B07 | GSAAPpYLKTKF  | 1229          | 1373         | 2717        | 3378      | 3557       | 1.9                  | 0.97           |
| 9   | B08 | GWMEDpYDYVHL  | 538           | 556          | 1385        | 1253      | 1362       | 1.3                  | 0.9            |
| 10  | B10 | HNSALpYSQVQK  | 616           | 599          | 1272        | 1535      | 1695       | 1.96                 | 0.97           |
| 11  | B11 | HRQLNpYIQVDL  | 357           | 119          | 691         | 984       | 1565       | 11.8                 | 0.95           |
| 12  | B14 | IEDNEpYTARQG  | 284           | 248          | 688         | 575       | 1003       | 3.2                  | 0.89           |
| 13  | B15 | IESDipYAEIPD  | 7398          | 6925         | 8638        | 8713      | 10095      | 0.61                 | 0.99           |
| 14  | B18 | IYETDpYYRKGG  | 532           | 361          | 868         | 850       | 1364       | 2.6                  | 0.88           |
| 15  | B20 | KDRMSpYHVRSH  | 763           | 935          | 1559        | 1523      | 2355       | 2                    | 0.94           |
| 16  | B21 | KKRCPpYTKHQT  | 656           | 715          | 998         | 1254      | 1609       | 1.6                  | 0.97           |
| 17  | B22 | KVVALpYDYMPM  | 495           | 679          | 1317        | 1170      | 1235       | 1                    | 0.92           |
| 18  | C06 | NNHTEpYASIQT  | 1837          | 1359         | 1872        | 1750      | 2480       | 0.23                 | 0.85           |
| 19  | C08 | NSDVQpYTEVQV  | 2645          | 4453         | 8099        | 7872      | 9583       | 1.6                  | 0.96           |
| 20  | C11 | PEGLNpYACLTH  | 654           | 1134         | 1667        | 2067      | 2371       | 1.9                  | 0.99           |
| 21  | C12 | PEGHEpYpYRVRE | 570           | 186          | 1305        | 1593      | 2158       | 5.5                  | 0.92           |
| 22  | C14 | PGSLEpYLCLPA  | 388           | 436          | 911         | 1156      | 2047       | 9.6                  | 0.97           |
| 23  | C16 | PLDKDpYpYVRE  | 248           | 241          | 653         | 666       | 1013       | 3.8                  | 0.95           |
| 24  | C19 | PSFSEpYASVQV  | 2319          | 3650         | 3576        | 3659      | 3564       | 0.29                 | 0.96           |
| 25  | C22 | QGPVI pYAQLDH | 609           | 662          | 1069        | 1227      | 1095       | 0.79                 | 0.95           |
| 26  | C23 | QQQPIpYIVMEL  | 2676          | 2700         | 3809        | 5075      | 6003       | 1.48                 | 0.96           |
| 27  | C24 | QQQEVpYGMMPR  | 628           | 511          | 890         | 1260      | 1902       | 4.7                  | 0.94           |
| 28  | D04 | SDDVRpYVNAFK  | 341           | 692          | 1207        | 1620      | 1660       | 2.37                 | 0.98           |
| 29  | D07 | SESVVpYADIRK  | 5294          | 4930         | 3902        | 6636      | 7119       | 0.29                 | 0.97           |
| 30  | D18 | TSSVLpYTAVQP  | 7232          | 7598         | 8160        | 8157      | 12415      | 0.43                 | 0.88           |
| 31  | D20 | VDTPHpYPRWIT  | 999           | 2026         | 2378        | 2933      | 2735       | 0.97                 | 0.97           |
| 32  | D21 | VNTTLpYEKFTY  | 5664          | 9184         | 7019        | 7582      | 10414      | 0.55                 | 0.93           |
| 33  | D23 | YETDYpYRKGGK  | 260           | 160          | 713         | 1153      | 2208       | 62                   | 0.98           |
| 34  | D24 | YFMTEpYVATRW  | 2635          | 2877         | 3924        | 4383      | 5359       | 0.92                 | 0.97           |
| 35  | E06 | AKIQDpYHILTR  | 395           | 478          | 1074        | 1053      | 1535       | 2.7                  | 0.95           |
| 36  | E09 | ATVGHpYTAVQN  | 672           | 1187         | 1101        | 1363      | 1431       | 0.62                 | 0.96           |
| 37  | E10 | AYRQLpYLNPKG  | 467           | 329          | 1210        | 1725      | 2329       | 6.6                  | 0.97           |
| 38  | E12 | DINSLpYDVSRM  | 2275          | 2652         | 2756        | 3319      | 5096       | 1.12                 | 0.88           |
| 39  | E18 | ELGYEpYMDVGS  | 206           | 253          | 416         | 666       | 1285       | 25.5                 | 0.98           |
| 40  | E19 | AELEFpYMDYEA  | 10862         | 14112        | 18376       | 17783     | 19714      | 0.54                 | 0.99           |
| 41  | E21 | GPQDipYDVPPV  | 5884          | 5325         | 6566        | 6644      | 10036      | 0.54                 | 0.89           |
| 42  | E24 | IGEGTpYGTVFK  | 444           | 686          | 1352        | 1375      | 1820       | 2.27                 | 0.97           |
| 43  | F01 | IYIHRpYENVSI  | 7054          | 8384         | 7719        | 9403      | 8592       | 0.16                 | 0.98           |
| 44  | F05 | LDSTFpYRSLL   | 361           | 347          | 1215        | 1312      | 1582       | 3                    | 0.93           |
| 45  | F08 | LMGHEpYMEMKN  | 635           | 1368         | 1512        | 1246      | 1484       | 0.51                 | 0.89           |
| 46  | F09 | LNKQGpYKCRQC  | 719           | 798          | 1234        | 1659      | 2677       | 0.52                 | 0.96           |
| 47  | F21 | VCAERpYSQEVF  | 135           | 462          | 979         | 1263      | 1719       | 5.3                  | 0.98           |

SHP1 Kd

| NO. | ID  | Sequence       | Fluorescence  |              |             |           |            | SHP1<br>Kd/ $\mu$ M | R <sup>2</sup> |
|-----|-----|----------------|---------------|--------------|-------------|-----------|------------|---------------------|----------------|
|     |     |                | 0.625 $\mu$ M | 1.25 $\mu$ M | 2.5 $\mu$ M | 5 $\mu$ M | 10 $\mu$ M |                     |                |
| 1   | A02 | AEMTGpYVVTRW   | 721           | 1136         | 2930        | 3222      | 3709       | 2.7                 | 0.95           |
| 2   | A04 | DEELHpYASLNF   | 4391          | 4557         | 6895        | 8504      | 9318       | 1.18                | 0.98           |
| 3   | A05 | DEGIHpYSELIQ   | 1229          | 1271         | 4311        | 7416      | 7501       | 4.8                 | 0.93           |
| 4   | A09 | DKQVEpYLDLDL   | 1188          | 1974         | 2507        | 4771      | 5777       | 4.7                 | 0.98           |
| 5   | A11 | DTETVpYSEVRK   | 6289          | 9680         | 10849       | 13491     | 15583      | 1                   | 0.99           |
| 6   | A13 | EDEDYpYKASVT   | 370           | 305          | 453         | 895       | 1558       | 19                  | 0.96           |
| 7   | A15 | EDGISpYTTLRF   | 5914          | 6588         | 9232        | 13551     | 14995      | 1.83                | 0.98           |
| 8   | A16 | EDSTYpYKASKG   | 200           | 66           | 896         | 1066      | 1337       | 5.7                 | 0.9            |
| 9   | A17 | EDTLTpYADLDM   | 2020          | 3399         | 3511        | 8349      | 11035      | 7.9                 | 0.97           |
| 10  | A18 | EGVATpYAAAVL   | 3756          | 2849         | 5343        | 6910      | 6183       | 0.96                | 0.9            |
| 11  | A19 | EGDNDpYIPLP    | 1467          | 2252         |             | 5387      | 5853       | 2.63                | 0.99           |
| 12  | A21 | EPNVSpYICSR    | 4390          | 2853         | 7943        | 11590     | 12385      | 3                   | 0.94           |
| 13  | A22 | ESDGSpYQKPSY   | 222           | 105          | 1117        | 1032      | 1486       | 5                   | 0.87           |
| 14  | B03 | FLFNMpYLTRER   | 1224          | 806          | 1442        | 2850      | 3518       | 5.2                 | 0.93           |
| 15  | B04 | FMMTPpYVVTRY   | 2988          | 4939         | 6899        | 8327      | 8934       | 1.39                | 0.99           |
| 16  | B05 | GFLTEpYVATRW   | 2865          | 4328         | 3100        | 8140      | 9960       | 4.4                 | 0.89           |
| 17  | B07 | GSAAPpYLKTKF   | 2883          | 4957         | 5185        | 7849      | 10693      | 2.8                 | 0.96           |
| 18  | B10 | HNSALpYSQVQK   | 824           | 1528         | 3507        | 5046      | 6208       | 5                   | 0.99           |
| 19  | B11 | HRQLNpYIQVDL   | 1532          | 1501         | 2575        | 3612      | 3274       | 1.4                 | 0.94           |
| 20  | B15 | IESDlpYAEIPD   | 12278         | 15308        | 14846       | 15241     | 17145      | 0.2                 | 0.98           |
| 21  | B17 | IRYHRpYHGSA    | 1197          | 1723         | 2637        | 5657      | 7135       | 7.7                 | 0.98           |
| 22  | B18 | IYETDpYRKGK    | 1198          | 1220         | 3086        | 4317      | 3660       | 2.1                 | 0.9            |
| 23  | B19 | KAVDGpYVKPQI   | 1041          | 1099         | 1799        | 2343      | 2695       | 1.83                | 0.98           |
| 24  | B20 | KDRMSpYHVRS    | 1054          | 1741         | 2660        | 4979      | 4393       | 2.8                 | 0.93           |
| 25  | B21 | KKRCRpYTKHQT   | 1056          | 1105         | 1612        | 3460      | 4976       | 10.5                | 0.98           |
| 26  | C02 | LPPEGpYVVVVK   | 177           | 167          | 744         | 1142      | 1094       | 4.1                 | 0.9            |
| 27  | C06 | NNHTEpYASIQ    | 4511          | 5397         | 10011       | 11585     | 12301      | 1.6                 | 0.98           |
| 28  | C11 | PEGLNpYACLTH   | 5264          | 8216         | 9806        | 17618     | 20011      | 3.14                | 0.98           |
| 29  | C14 | PGSLEpYLCLPA   | 496           | 589          | 2317        | 2067      | 3475       | 5.4                 | 0.91           |
| 30  | C19 | PSFSEpYASVQV   | 4398          | 5876         | 4638        | 7367      | 9409       | 0.93                | 0.88           |
| 31  | C21 | PQDKEpYpYKVKE  | 304           | 396          | 710         | 811       | 1692       | 14                  | 0.95           |
| 32  | C22 | QGPVIpYpYALDH  | 5963          | 9279         | 13766       | 15334     | 16961      | 1.3                 | 0.99           |
| 33  | C24 | QQQEVpYpYGMMP  | 596           | 837          | 1879        | 2653      | 2604       | 2.7                 | 0.95           |
| 34  | D01 | RNEGVpYTAIAV   | 1158          | 1106         | 2124        | 2871      | 2973       | 1.92                | 0.97           |
| 35  | D02 | REGLNpYpYMLAT  | 3357          | 5667         | 9234        | 10729     | 13411      | 2.2                 | 0.99           |
| 36  | D04 | SDDVRpYpYVNAFK | 2332          | 3147         | 5604        | 7826      | 7820       | 2.14                | 0.98           |
| 37  | D05 | SDGHEpYpYIVDP  | 539           | 531          | 1052        | 1395      | 1775       | 3.1                 | 0.98           |
| 38  | D07 | SESVVpYpYADIRK | 13217         | 17579        | 23890       | 23521     | 18882      | 0.38                | 0.92           |
| 39  | D08 | SETDDpYpYAEIID | 1495          | 2122         | 3224        | 3497      | 2870       | 0.72                | 0.93           |
| 40  | D12 | SSTVQpYpYSTVVH | 7845          | 9834         | 15223       | 13923     | 15393      | 0.69                | 0.97           |
| 41  | D18 | TSSVLpYpYTAVQP | 11849         | 19048        | 21835       | 20480     | 18796      | 0.34                | 0.94           |
| 42  | D20 | VDTPHpYpYPRWIT | 2090          | 2926         | 4984        | 6152      | 6837       | 1.93                | 0.99           |
| 43  | D21 | VNTTLpYpYEKFTY | 1932          | 1884         | 3749        | 3263      | 4103       | 0.99                | 0.92           |
| 44  | D23 | YETDpYpYRKGGK  | 1310          | 1651         | 3636        | 3609      | 5467       | 3.1                 | 0.95           |
| 45  | D24 | YFMTEpYpYVATRW | 4074          | 6285         | 8710        | 9159      | 11042      | 1.15                | 0.99           |
| 46  | E04 | AFGTVpYpYKGIWI | 1734          | 2258         | 4509        | 5185      | 6632       | 2.6                 | 0.98           |
| 47  | E05 | AFQFSpYpYTAVFG | 1442          | 1888         | 3484        | 4176      | 6666       | 5                   | 0.97           |
| 48  | E06 | AKIQDpYpYHILTR | 1057          | 1314         | 2966        | 4938      | 4178       | 2.7                 | 0.91           |
| 49  | E07 | APAEMpYpYDIMKT | 286           | 347          | 932         | 926       | 1044       | 2                   | 0.93           |

SHP1 Kd

|    |     |               |      |      |       |      |      |      |      |
|----|-----|---------------|------|------|-------|------|------|------|------|
| 50 | E09 | ATVGHpYTAVQN  | 1932 | 3089 | 2679  | 5763 | 6572 | 3    | 0.93 |
| 51 | E10 | AYRQLpYLNPKG  | 1984 | 4011 | 6159  | 7679 | 8356 | 1.96 | 0.99 |
| 52 | E11 | CPEKVpYELMRA  | 979  | 1241 | 2014  | 2687 | 4193 | 5.2  | 0.98 |
| 53 | E12 | DINSLpYDVSRM  | 1297 | 1946 | 2507  | 2975 | 4119 | 1.9  | 0.97 |
| 54 | E13 | DRFIQpYANPAF  | 2731 | 3926 | 6075  | 6840 | 8594 | 1.8  | 0.99 |
| 55 | E15 | EDIKSpYYTVRQ  | 1986 | 2951 | 4059  | 6116 | 6634 | 2.2  | 0.99 |
| 56 | E18 | ELGYEpYMDVGS  | 2927 | 5289 | 4503  | 5830 | 7308 | 0.84 | 0.93 |
| 57 | E19 | AELEFpYMDYEA  | 5672 | 7643 | 10294 | 8335 | 9624 | 0.41 | 0.95 |
| 58 | E21 | GPQDIpYDVPPV  | 2304 | 2960 | 5825  | 4170 | 6346 | 1.1  | 0.87 |
| 59 | E24 | IGEGTpYGTVEFK | 2451 | 3439 | 5631  | 5876 | 5814 | 1    | 0.97 |
| 60 | F04 | LARDMpYDKEYY  | 236  | 438  | 876   | 798  | 880  | 1.4  | 0.92 |
| 61 | F07 | LLANApYIYVVQ  | 798  | 1025 | 2118  | 2521 | 3225 | 3    | 0.98 |
| 62 | F08 | LMGHEpYMEMKN  | 3312 | 3891 | 8491  | 9449 | 7800 | 1.2  | 0.89 |
| 63 | F10 | NKPTVpYGVSPN  | 611  | 608  | 1282  | 2617 | 3722 | 12   | 0.99 |
| 64 | F13 | PEPGPpYAQPSV  | 375  | 605  | 1530  | 1507 | 1529 | 1.75 | 0.9  |
| 65 | F16 | SADHLpYVNVSE  | 3300 | 5325 | 3874  | 5430 | 4586 | 0.22 | 0.89 |
| 66 | F22 | VDSSLpYNLPRS  | 302  | 547  | 1039  | 1021 | 2394 | 19   | 0.93 |

SHP2 Kd

| NO. | ID  | Sequence      | Fluorescence |               |              |             |           | SHP2<br>Kd/ $\mu$ M | R2   |
|-----|-----|---------------|--------------|---------------|--------------|-------------|-----------|---------------------|------|
|     |     |               | 0.31 $\mu$ M | 0.625 $\mu$ M | 1.25 $\mu$ M | 2.5 $\mu$ M | 5 $\mu$ M |                     |      |
| 1   | A01 | AEKPFpYVNVEF  | 1556         | 2228          | 4102         | 7112        | 5983      | 1.27                | 0.92 |
| 2   | A02 | AEMTGpYVVTRW  | 2318         | 5151          | 4485         | 8993        | 7347      | 0.74                | 0.87 |
| 3   | A04 | DEELHpYASLNF  | 5624         | 6511          | 9549         | 11238       | 9743      | 0.35                | 0.96 |
| 4   | A05 | DEGIHpYSELIQ  | 4308         | 4420          | 5928         | 8620        | 9464      | 0.73                | 0.96 |
| 5   | A06 | DEKVDpYVQVDK  | 1587         | 2545          | 4294         | 5952        | 4295      | 0.65                | 0.88 |
| 6   | A07 | DERVDpYVVVDQ  | 3134         | 4518          | 6176         | 7287        | 7813      | 0.56                | 0.99 |
| 7   | A09 | DKQVEpYLDLDL  | 7029         | 7244          | 12037        | 16120       | 14342     | 0.59                | 0.95 |
| 8   | A11 | DTETVpYSEVRK  | 5482         | 6515          | 8574         | 12778       | 14106     | 0.88                | 0.97 |
| 9   | A15 | EDGISpYTTLRF  | 6317         | 11017         | 9579         | 13809       | 13946     | 0.37                | 0.95 |
| 10  | A16 | EDSTYpYKASKG  | 1329         | 2260          | 2117         | 4507        | 3865      | 0.9                 | 0.89 |
| 11  | A17 | EDTLTpYADLDM  | 5184         | 5758          | 8286         | 16533       | 12726     | 0.96                | 0.86 |
| 12  | A18 | EGVATpYAAAVL  | 1595         | 1761          | 3919         | 5489        | 5227      | 1.1                 | 0.95 |
| 13  | A19 | EGDNDpYIPLP   | 2731         | 3693          | 5971         | 8655        | 7386      | 0.77                | 0.94 |
| 14  | A20 | ENGLNpYIDLDL  | 7833         | 9319          | 13166        | 12564       | 10987     | 0.18                | 0.94 |
| 15  | A21 | EPNVSpYICSTRY | 5579         | 6737          | 10468        | 13104       | 12301     | 0.56                | 0.97 |
| 16  | A22 | ESDGSpYQKPSY  | 991          | 1399          | 2075         | 3903        | 3059      | 1                   | 0.89 |
| 17  | B03 | FLFNMpYLTRER  | 3319         | 4328          | 5263         | 7506        | 5315      | 0.31                | 0.89 |
| 18  | B04 | FMMTPpYVVTRY  | 5828         | 7344          | 10740        | 15332       | 16175     | 0.91                | 0.99 |
| 19  | B07 | GSAAPpYLKTKF  | 5008         | 6916          | 9897         | 13490       | 13858     | 0.79                | 0.99 |
| 20  | B10 | HNSALpYSQVQK  | 2845         | 3317          | 6557         | 10028       | 10614     | 1.52                | 0.98 |
| 21  | B11 | HRQLNpYIQVDL  | 9137         | 11844         | 15587        | 17671       | 15023     | 0.27                | 0.96 |
| 22  | B17 | IRYHRpYHGRSA  | 4238         | 5329          | 8634         | 14012       | 11863     | 0.93                | 0.93 |
| 23  | B19 | KAVDGpYVKPQI  | 5588         | 8763          | 11789        | 14813       | 11818     | 0.41                | 0.94 |
| 24  | B20 | KDRMSpYHVRSH  | 2603         | 3077          | 5023         | 9595        | 9154      | 1.6                 | 0.94 |
| 25  | B21 | KKRCPpYTKHQT  | 3243         | 4930          | 7467         | 12491       | 9319      | 0.82                | 0.89 |
| 26  | B22 | KVVALpYDYMPM  | 2087         | 3667          | 5414         | 5005        | 5649      | 0.46                | 0.96 |
| 27  | C02 | LPPEGpYVVVVK  | 2929         | 3208          | 3695         | 8136        | 8993      | 1.86                | 0.93 |
| 28  | C06 | NNHTEpYASIQT  | 6977         | 8274          | 11913        | 14255       | 14359     | 0.47                | 0.99 |
| 29  | C09 | NSKRDpYTGCT   | 794          | 891           | 982          | 3231        | 4264      | 6.9                 | 0.95 |
| 30  | C11 | PEGLNpYACLTH  | 10471        | 13154         | 20255        | 19219       | 17550     | 0.27                | 0.94 |
| 31  | C14 | PGSLEpYLCLPA  | 2145         | 4050          | 6753         | 6992        | 7496      | 0.69                | 0.97 |
| 32  | C15 | PKGTGpYIKTEL  | 2293         | 2578          | 3123         | 7591        | 7499      | 1.9                 | 0.91 |
| 33  | C19 | PSFSEpYASVQV  | 4757         | 5225          | 8118         | 10667       | 10429     | 0.62                | 0.97 |
| 34  | C22 | QGPVIpYAQLDH  | 13661        | 13753         | 20153        | 20918       | 18023     | 0.18                | 0.95 |
| 35  | C23 | QKQPIpYIVMEL  | 2812         | 3486          | 4697         | 10920       | 9186      | 1.47                | 0.88 |
| 36  | D01 | RNEGVpYTAIAV  | 3272         | 3859          | 6148         | 10614       | 8540      | 0.91                | 0.9  |
| 37  | D02 | REGLNpYMLAT   | 7230         | 10217         | 13107        | 18710       | 14516     | 0.45                | 0.93 |
| 38  | D04 | SDDVRpYVNAFK  | 5892         | 7428          | 11980        | 16996       | 12470     | 0.56                | 0.89 |
| 39  | D07 | SESVVpYADIRK  | 11697        | 17607         | 19078        | 19696       | 16610     | 0.15                | 0.95 |
| 40  | D12 | SSTVQpYSTVWH  | 11828        | 15822         | 21067        | 18167       | 15428     | 0.13                | 0.9  |
| 41  | D16 | TNDITpYADLNL  | 9679         | 10511         | 13243        | 14859       | 10472     | 0.11                | 0.91 |
| 42  | D18 | TSSVLpYTAVQP  | 17406        | 19396         | 23935        | 20409       | 18569     | 0.03                | 0.91 |
| 43  | D23 | YETDYPYRKGGK  | 2180         | 3173          | 4520         | 8610        | 6658      | 1                   | 0.88 |
| 44  | D24 | YFMTEpYVATRW  | 4243         | 4262          | 7505         | 8230        | 6160      | 0.29                | 0.87 |
| 45  | E04 | AFGTVpYKGIWI  | 10571        | 12366         | 18142        | 19582       | 18830     | 0.33                | 0.98 |
| 46  | E05 | AFQFSpYTAVFG  | 1007         | 2899          | 7919         | 7158        | 6228      | 1.57                | 0.86 |
| 47  | E06 | AKIQDpYHILTR  | 3641         | 4564          | 5715         | 11772       | 8799      | 0.86                | 0.85 |
| 48  | E09 | ATVGHpYTAVQN  | 5839         | 6293          | 14251        | 21383       | 19527     | 1.22                | 0.93 |
| 49  | E10 | AYRQLpYLNPKG  | 4345         | 4892          | 8051         | 10858       | 15176     | 1.8                 | 0.98 |

SHP2 Kd

|    |     |               |      |      |       |       |       |      |      |
|----|-----|---------------|------|------|-------|-------|-------|------|------|
| 50 | E13 | DRFIQpYANPAF  | 5894 | 7120 | 10413 | 10647 | 12829 | 0.46 | 0.98 |
| 51 | E15 | EDIKSpYYTVRQ  | 4627 | 5156 | 8336  | 6393  | 6933  | 0.18 | 0.9  |
| 52 | E18 | ELGYEpYMDVGS  | 3999 | 5733 | 7016  | 6829  | 9942  | 0.49 | 0.95 |
| 53 | E22 | GQSESpYGNITY  | 2462 | 2450 | 3730  | 5418  | 6013  | 0.94 | 0.96 |
| 54 | E24 | IGEGTpYGTVEFK | 6737 | 7053 | 9844  | 13000 | 13023 | 0.5  | 0.97 |
| 55 | F01 | IYIHRpYENVSI  | 4991 | 6937 | 8745  | 7623  | 7615  | 0.17 | 0.95 |
| 56 | F05 | LDSTFpYRSLLE  | 2345 | 3057 | 5043  | 6812  | 5129  | 0.55 | 0.9  |
| 57 | F06 | LGQRIpYQYIQS  | 5194 | 6363 | 10355 | 9126  | 8983  | 0.28 | 0.93 |
| 58 | F08 | LMGHEpYMEMKN  | 3088 | 3545 | 5278  | 4613  | 6100  | 0.35 | 0.95 |
| 59 | F13 | PEPGPpYQAQPSV | 1077 | 864  | 2074  | 2856  | 2900  | 1.15 | 0.94 |
| 60 | F15 | RNPGFpYVEANP  | 1212 | 1550 | 4374  | 5540  | 5138  | 1.2  | 0.91 |
| 61 | F16 | SADHLpYVNVSE  | 1152 | 1670 | 3093  | 2654  | 3751  | 0.76 | 0.94 |
| 62 | F21 | VCAERpYSQEVF  | 2566 | 4561 | 6184  | 12727 | 10341 | 1.28 | 0.9  |
| 63 | F22 | VDSSLpYNLPRS  | 877  | 1481 | 3346  | 3094  | 3820  | 0.97 | 0.93 |
| 64 | F23 | VSSTHpYYLLPE  | 2078 | 2792 | 4928  | 7642  | 7209  | 1.2  | 0.96 |

dual color

| NO | ID  | Peptide sequence | Log <sub>2</sub> (protein 1/protein 2) |                       |                            |
|----|-----|------------------|----------------------------------------|-----------------------|----------------------------|
|    |     |                  | PTP1B/TCPTP                            | SHP1/SHP2 full length | SHP1/SHP2 catalytic domain |
| 1  | A01 | AEKPFpYVNVEF     | -0.38                                  | 1.20                  | 0.95                       |
| 2  | A02 | AEMTGpYVVTRW     | 0.11                                   | 1.32                  | 0.80                       |
| 3  | A03 | AENAEpYLRVAP     | -0.18                                  | 0.60                  | 1.04                       |
| 4  | A04 | DEELHpYASLNF     | 0.00                                   | 0.42                  | 0.71                       |
| 5  | A05 | DEGIHpYSELIQ     | -0.43                                  | 1.33                  | 0.70                       |
| 6  | A06 | DEKVDpYVQVDK     | -0.69                                  | 0.19                  | 0.74                       |
| 7  | A07 | DERVDpYVVVDQ     | -0.10                                  | 0.31                  | -0.49                      |
| 8  | A08 | DESVDpYVPMLD     | -0.07                                  | 0.73                  | 1.18                       |
| 9  | A09 | DKQVEpYLDLDDL    | 0.01                                   | -0.06                 | 0.69                       |
| 10 | A10 | DSGGFpYITSRT     | 0.41                                   | 0.63                  | 0.12                       |
| 11 | A11 | DTETVpYSEVRK     | 0.26                                   | 1.64                  | 0.06                       |
| 12 | A12 | EANSHpYGHND      | 0.25                                   | -0.45                 | -0.69                      |
| 13 | A13 | EDEDYpYKASVT     | -0.34                                  | 0.67                  | 0.15                       |
| 14 | A14 | EDGGVpYSSSGL     | 0.12                                   | 0.28                  | 0.30                       |
| 15 | A15 | EDGISpYTTLRF     | 0.12                                   | 0.55                  | -0.17                      |
| 16 | A16 | EDSTYpYKASKG     | 0.18                                   | 0.48                  | 0.58                       |
| 17 | A17 | EDTLTpYADLDM     | -0.45                                  | 0.15                  | -0.17                      |
| 18 | A18 | EGVATpYAAAVL     | 0.24                                   | 3.23                  | 2.91                       |
| 19 | A19 | EGDNDpYIPLP      | -0.06                                  | 0.11                  | -1.36                      |
| 20 | A20 | ENGLNpYIDLDDL    | 0.10                                   | -0.62                 | 0.48                       |
| 21 | A21 | EPNVSpYICSR      | 0.75                                   | 1.65                  | 0.12                       |
| 22 | A22 | ESDGSpYQKPSY     | 0.32                                   | 0.12                  | 0.20                       |
| 23 | A23 | ESDGGpYMDMSK     | 0.01                                   | -0.12                 | -1.69                      |
| 24 | A24 | ETDKEpYYTVKD     | 0.26                                   | 0.11                  | 0.20                       |
| 25 | B01 | FEEDDpYESPND     | -0.62                                  | 1.12                  | 0.06                       |
| 26 | B02 | FGMTRpYVLDDE     | -0.22                                  | -0.81                 | -0.27                      |
| 27 | B03 | FLFNMpYLTRER     | 1.04                                   | 0.56                  | 0.04                       |
| 28 | B04 | FMMTPpYVVTRY     | 1.28                                   | 1.06                  | -0.12                      |
| 29 | B05 | GFLTEpYVATRW     | 0.53                                   | 1.29                  | 0.44                       |
| 30 | B06 | GNNYVpYIDPTQ     | 1.24                                   | 2.40                  | 1.97                       |
| 31 | B07 | GSAAPpYLKTKF     | 1.71                                   | 0.60                  | 0.10                       |
| 32 | B08 | GWMEDpYDYVHL     | 1.26                                   | 1.17                  | 0.44                       |
| 33 | B09 | GWMVHpYTSKDT     | 0.12                                   | -0.23                 | -0.12                      |
| 34 | B10 | HNSALpYSQVQK     | 0.20                                   | 0.26                  | -0.20                      |
| 35 | B11 | HRQLNpYIQVDL     | 0.80                                   | -1.03                 | 0.01                       |
| 36 | B12 | IEDNEpYTAREG     | 0.75                                   | -0.89                 | -0.69                      |
| 37 | B13 | IEDEDpYYKASV     | 0.77                                   | 0.74                  | 0.20                       |
| 38 | B14 | IEDNEpYTARQG     | 0.57                                   | 0.25                  | -0.07                      |
| 39 | B15 | IESDlpYAEIPD     | 1.64                                   | 4.76                  | 2.16                       |
| 40 | B16 | IESSNpYMAPYD     | 0.07                                   | 0.11                  | -0.43                      |
| 41 | B17 | IRYHRpYHGRSA     | 0.74                                   | 0.60                  | -0.18                      |
| 42 | B18 | IYETDpYYRKGG     | 0.06                                   | 0.78                  | -0.18                      |
| 43 | B19 | KAVDGpYVKPQI     | 0.34                                   | -0.54                 | 0.03                       |
| 44 | B20 | KDRMSpYHVRSH     | 1.29                                   | 0.19                  | -0.43                      |
| 45 | B21 | KKRCPpYTKHQT     | 0.77                                   | 0.43                  | -0.20                      |
| 46 | B22 | KVVALpYDYMPM     | 0.93                                   | 0.32                  | 0.16                       |
| 47 | B23 | LISSDpYELLSD     | 0.56                                   | 0.23                  | -0.10                      |
| 48 | B24 | LNSDGpYTPEPA     | -0.22                                  | 0.12                  | 0.01                       |

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|    |     |                |       |       |       |
|----|-----|----------------|-------|-------|-------|
| 49 | C01 | LNSKGpYTKSID   | -0.47 | -1.15 | -0.54 |
| 50 | C02 | LPPEGpYVVVVVK  | 1.12  | -0.27 | 0.15  |
| 51 | C03 | MDTSVpYESPYS   | -0.29 | -0.64 | -0.89 |
| 52 | C04 | MKDEEpYEQMVK   | -0.14 | -0.60 | -0.12 |
| 53 | C05 | MTGDTpYTAHAG   | -0.40 | 0.30  | -0.67 |
| 54 | C06 | NNHTEpYASIQT   | 0.96  | 1.30  | 1.01  |
| 55 | C07 | NQSSGpYRYGTD   | -0.51 | -0.81 | -1.06 |
| 56 | C08 | NSDVQpYTEVQV   | 0.84  | 1.91  | 2.09  |
| 57 | C09 | NSKRDpYTGCS    | 0.20  | -0.17 | 0.01  |
| 58 | C10 | NVVPLpYDLLLE   | 0.20  | 0.42  | 0.07  |
| 59 | C11 | PEGLNpYACLTH   | 0.31  | 0.06  | 0.23  |
| 60 | C12 | PEGHEpYpYRVRE  | -0.43 | 1.01  | -0.27 |
| 61 | C13 | PEQDEpYDIPRH   | -1.06 | -0.18 | -0.27 |
| 62 | C14 | PGSLEpYLCLPA   | 0.34  | 0.50  | 0.96  |
| 63 | C15 | PKGTGpYIKTEL   | 0.58  | -0.01 | -0.29 |
| 64 | C16 | PLDKDpYpYVVRE  | -0.10 | 0.65  | 0.06  |
| 65 | C17 | PPDHQpYYNDFP   | 0.20  | -0.17 | 0.00  |
| 66 | C18 | PQDKEpYKVKKE   | -0.60 | 0.00  | -0.58 |
| 67 | C19 | PSFSEpYASVQV   | 1.54  | 2.10  | 2.11  |
| 68 | C20 | PSTRDpYEIQRE   | -0.58 | -0.43 | -0.51 |
| 69 | C21 | PQDKEpYpYKVKKE | 0.93  | -0.14 | -0.03 |
| 70 | C22 | QGPVI pYAQLDH  | 0.33  | 0.01  | 0.41  |
| 71 | C23 | QKQPIpYIVMEL   | 1.16  | 1.17  | 0.43  |
| 72 | C24 | QQQEVpYGMMPR   | -0.07 | 0.37  | -0.10 |
| 73 | D01 | RNEGVpYTAIAV   | 0.68  | 1.22  | 0.48  |
| 74 | D02 | REGLNpYMLLAT   | -0.01 | 0.00  | -0.01 |
| 75 | D03 | SAEPQpYQPGDQ   | -0.36 | -0.64 | -0.15 |
| 76 | D04 | SDDVRpYVNAFK   | 0.76  | 0.21  | -0.07 |
| 77 | D05 | SDGHEpYIYVDP   | 0.52  | -0.23 | 0.28  |
| 78 | D06 | SEEPipYIVTEY   | 0.53  | 1.01  | 1.20  |
| 79 | D07 | SESVVpYADIRK   | 1.26  | 1.67  | 0.42  |
| 80 | D08 | SETDDpYAEIID   | 0.86  | 0.01  | 0.62  |
| 81 | D09 | SPPALpYAEPLD   | -0.60 | -0.60 | 0.43  |
| 82 | D10 | SSNPEpYLSASD   | 0.01  | 0.20  | -0.42 |
| 83 | D11 | SSSSEpYGSVSP   | -0.34 | -0.12 | -0.27 |
| 84 | D12 | SSTVQpYSTVVH   | 1.69  | 1.35  | 0.96  |
| 85 | D13 | STEPQpYQPGEN   | 0.01  | 0.16  | -0.03 |
| 86 | D14 | TDKEYpYTVKDD   | 0.40  | 0.37  | 0.01  |
| 87 | D15 | TGGSVpYTEDND   | -0.25 | -0.47 | -0.42 |
| 88 | D16 | TNDITpYADLNL   | 0.37  | 0.38  | 0.99  |
| 89 | D17 | TSKLI pYDFIED  | 0.55  | -0.18 | 0.38  |
| 90 | D18 | TSSVLpYTAVQP   | 0.89  | 1.32  | 0.86  |
| 91 | D19 | VDADepYLIPQQ   | 0.29  | 0.21  | 0.19  |
| 92 | D20 | VDTPHpYPRWIT   | 1.66  | 1.27  | 0.04  |
| 93 | D21 | VNTTLpYEKFTY   | 1.39  | 0.71  | 0.14  |
| 94 | D22 | VYESPpYSDPEE   | 0.82  | 1.23  | 0.25  |
| 95 | D23 | YETDYpYRKGGK   | 0.58  | 0.25  | -0.23 |
| 96 | D24 | YFMTEpYVATRW   | 1.73  | 2.12  | 0.57  |
| 97 | E01 | AEGSApYEEVPT   | -0.10 | -0.71 | -0.27 |
| 98 | E02 | AENPEpYLSEFS   | -0.69 | -0.15 | -0.45 |
| 99 | E03 | FDNLYpYWDQDP   | 0.25  | 0.01  | -0.25 |

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|     |     |               |       |       |       |
|-----|-----|---------------|-------|-------|-------|
| 100 | E04 | AFGTVpYKGIWI  | 0.15  | 0.72  | 0.10  |
| 101 | E05 | AFQFSpYTAVFG  | 0.01  | 0.57  | -0.04 |
| 102 | E06 | AKIQDpYHILTR  | 0.20  | 0.30  | -0.20 |
| 103 | E07 | APAEMpYDIMKT  | 0.11  | -0.58 | -0.36 |
| 104 | E08 | ASEQGpYEEMRA  | 0.36  | 0.15  | -0.62 |
| 105 | E09 | ATVGHpYTAVQN  | 0.45  | -0.34 | 0.07  |
| 106 | E10 | AYRQLpYLNPKG  | 0.59  | 0.16  | -0.07 |
| 107 | E11 | CPEKVpYELMRA  | -0.18 | -0.15 | -0.32 |
| 108 | E12 | DINSLpYDVSRM  | -0.01 | 0.77  | -0.12 |
| 109 | E13 | DRFIQpYANPAF  | 0.18  | -0.40 | -0.09 |
| 110 | E14 | DVSRMpYVDPSE  | 0.33  | 1.43  | 0.40  |
| 111 | E15 | EDIKSpYYTVRQ  | 0.65  | 0.03  | -0.25 |
| 112 | E16 | EEIRFpYQLGEE  | -0.29 | -0.22 | -1.03 |
| 113 | E17 | EKIQDpYEKMPE  | -0.22 | -2.94 | -0.60 |
| 114 | E18 | ELGYEpYMDVGS  | -0.56 | 1.33  | -0.40 |
| 115 | E19 | AELEFpYMDYEA  | 1.43  | 4.14  | 2.72  |
| 116 | E20 | FGAKPpYDGIPA  | 0.74  | -0.32 | -0.38 |
| 117 | E21 | GPQDIpYDVPPV  | 0.77  | 3.66  | 0.76  |
| 118 | E22 | GQSEpYGNITY   | -0.01 | 0.21  | -0.14 |
| 119 | E23 | GRETIpYPNASL  | 0.31  | -0.32 | -0.38 |
| 120 | E24 | IGEGTpYGTVFK  | 0.31  | 0.15  | 0.16  |
| 121 | F01 | IYIHRpYENVSI  | 0.50  | 1.01  | 1.07  |
| 122 | F02 | KAEDepYVNEPL  | -0.67 | -0.67 | -0.58 |
| 123 | F03 | KPKQEpYLNPE   | -0.38 | -0.76 | 0.45  |
| 124 | F04 | LARDMpYDKEYY  | 0.78  | 0.19  | -0.04 |
| 125 | F05 | LDSTFpYRSLLE  | 0.03  | -0.29 | -0.06 |
| 126 | F06 | LGQRIpYQYIQS  | 0.24  | 0.53  | 1.04  |
| 127 | F07 | LLANApYIYVVQ  | 0.77  | -0.34 | -0.38 |
| 128 | F08 | LMGHEpYMEMKN  | 0.55  | 1.17  | 0.33  |
| 129 | F09 | LNKQGpYKCRQC  | 0.31  | 1.06  | 0.39  |
| 130 | F10 | NKPTVpYGVSPN  | -1.00 | -0.17 | 0.50  |
| 131 | F11 | PPDHQpYYNDFP  | 0.41  | -0.38 | 0.60  |
| 132 | F12 | PEDTFpYFDPEF  | 0.39  | -0.10 | 0.37  |
| 133 | F13 | PEPGPpYQAQPSV | -1.84 | -0.56 | 0.49  |
| 134 | F14 | RHDSGpYEVHHQ  | 0.33  | 0.71  | 0.28  |
| 135 | F15 | RNPGFpYVEANP  | -0.67 | -1.29 | 0.10  |
| 136 | F16 | SADHLpYVNVSE  | 0.60  | 2.00  | 0.91  |
| 137 | F17 | SSDPTpYTSSLG  | -0.47 | 0.01  | 2.42  |
| 138 | F18 | EEEPVpYEAPE   | -0.74 | -0.29 | 0.61  |
| 139 | F19 | STKYFpYKQNGR  | -0.56 | -0.86 | 0.64  |
| 140 | F20 | TAEPDpYGALYE  | -0.14 | 0.01  | 0.21  |
| 141 | F21 | VCAERpYSQEVF  | -0.15 | 0.73  | 1.21  |
| 142 | F22 | VDSSLpYNLPRS  | 0.18  | 0.75  | 0.64  |
| 143 | F23 | VSSTHpYYLLPE  | 0.19  | -0.38 | 0.82  |
| 144 | F24 | VVIALpYDYQTN  | 0.21  | 0.50  | 0.77  |