Supplementary materials;

Supplementary Table 1. It summarizes the results of the Sequence Harmony analysis. Residues that are close to the binding pocket and determine specificity are shown.

Supplementary Figure 1. Experimental strategy. A) Structure of the phosphorylated peptide microarrays used to profile the human PTP domains substrate specificity. Three identical sub-arrays of 6,400 spots were printed on the chip surface. Each sub-array is formed by a 4x4 grid and each small grid unit contains phosphorylated peptides and control spots. B) Microarrays were incubated with the trapping mutants of Protein Tyrosine Phosphatases, expressed as GST fusion-proteins. The interaction signal was revealed by an anti-GST antibody conjugated to a fluorophore. C) The signal of each spot was measured with a laser scanner (Perkin Elmer) and peptides were ranked according to spot fluorescence intensity. The color of each spot is related to fluorescence intensity. Warm and cold colors indicate high and low fluorescence intensity respectively; black represents no signal.

Supplementary Figure 2. Kinetic analysis of phospho-peptide dephosphorylation. Purified enzyme, 1 μg/ml, was incubated with one of 10 phosphopeptides (100 μM) for 30 minutes. The release of phosphate was monitored every 5 minutes by incubating with Malachite Green.

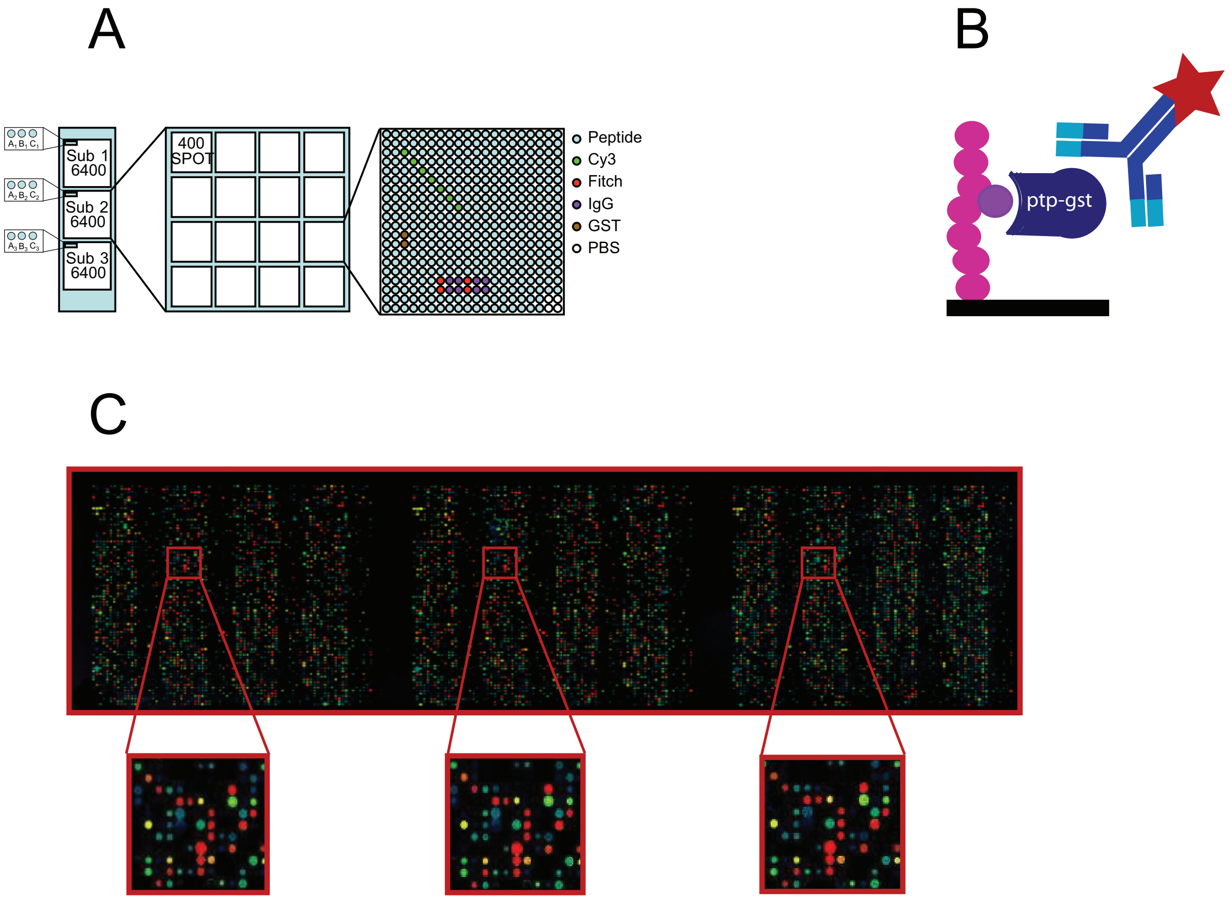
Supplementary File 1. Multisequence alignment of the phosphatase domains. The residues at position 59 are highlighted.

Supplementary spreadsheet 1. It is a table reporting the data of the chip experiments

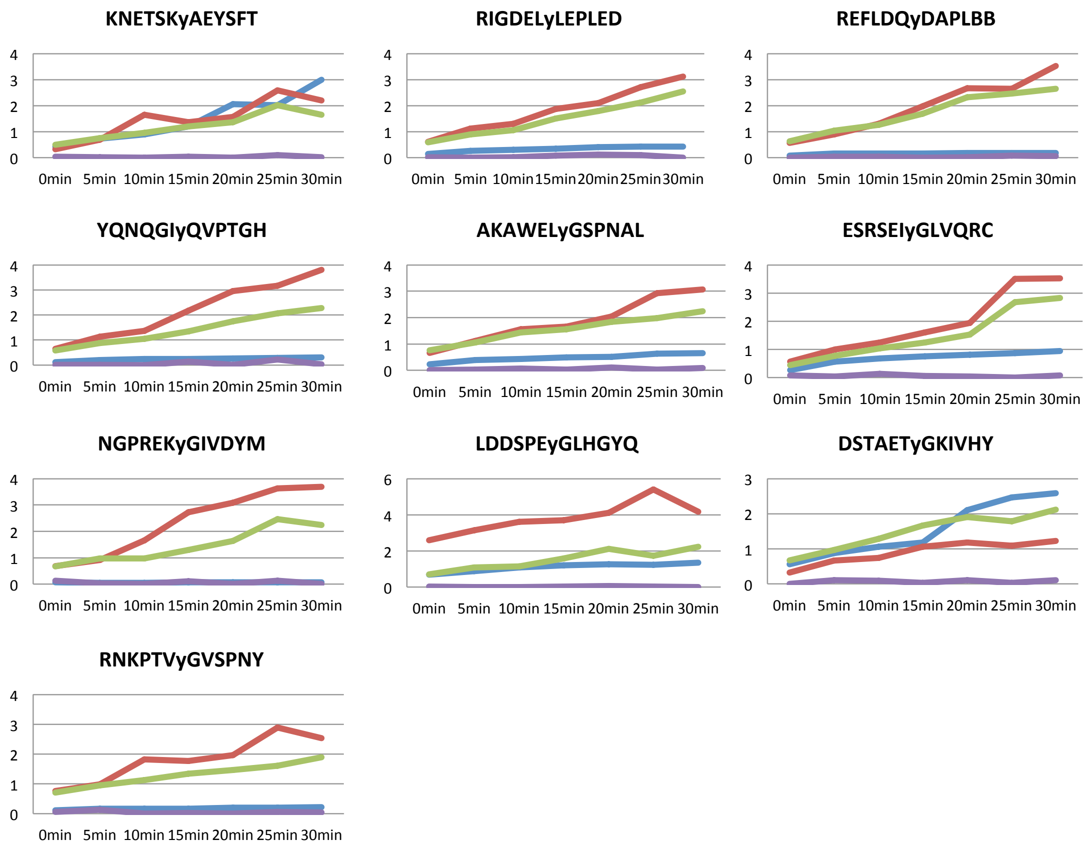
Supplementary Table 1

Alignment position	Diagnostic esidues	Sequence in PTP1B
54	R in group 5 K in other groups	KN**R**NR
59	D or N	YR**D**VS
306	L in group 5 and 7, Y in other groups	DQ**L**RF
309	V in group 3 S in group 5 I, V, L in other groups	RF**S**YL
312	Conserved A C in group 6	YL**A**VI

Supplementary Figure 1



Supplementary Figure 2



Supplementary file 1

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 Name: hPTPbeta_PTPRB oo  Len:  318  Check:  1819  Weight:  6.5
 Name: hSAP1_PTPRH oo  Len:  318  Check:   706  Weight:  7.1
 Name: hLAR_PTPRF oo  Len:  318  Check:  4133  Weight:  6.9
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 Name: hSHP1_PTPN6 oo  Len:  318  Check:  8310  Weight:  6.1
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 Name: hPTP1B_PTPN1 oo  Len:  318  Check:  9520  Weight:  6.2
 Name: hTCPTP_PTPN2 oo  Len:  318  Check:  6952  Weight:  6.1
 Name: hMEG2_PTPN9 oo  Len:  318  Check:  5562  Weight:  8.3
 Name: hMEG1_PTPN4 oo  Len:  318  Check:  4777  Weight:  6.5
 Name: hPTPH1_PTPN3 oo  Len:  318  Check:  3088  Weight:  6.7
 Name: hHDPTP_PTPN23 oo  Len:  318  Check:  7788  Weight:  8.2

//

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hPTPbeta_PTPRB        .........N QFEGHFMKLQ ADSNYLLSKE YEELKDVGRN QSCDIALLPE
hSAP1_PTPRH           .........E DFADHVRKNE RDSNCGFADE YQQLSLVGHS QSQMVASASE
hLAR_PTPRF            .........T DLADNIERLK ANDGLKFSQE YESIDPG.QQ FTWENSNLEV
hPTPalpha_PTPRA       .........D KLEEEINRRM ADDNKLFREE FNALPACPIQ ATCEAASKEE
hSHP1_PTPN6           .ADIENRVLE LNKKQESEDT AKAGFWEEFE SLQKQEVKNL HQRLEGQRPE
hSHP2_PTPN11          .AEIESRVRE LSKLAETTDK VKQGFWEEFE TLQQQECKLL YSRKEGQRQE
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hMEG1_PTPN4           ........HS LRESMIQLAE GLITGTVLTQ FDQLYRKKPG MTMSCAKLPQ
hPTPH1_PTPN3          ........DT LEGSMAQLKK GLESGTVLIQ FEQLYRKKPG LAITFAKLPQ
hHDPTP_PTPN23         ..ERLRQLQQ ELEAFRGQLG DVGALDTVWR ELQDAQEHDA RGRSIAIARC

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hLAR_PTPRF            NKPKNRYANV IAYDHSRVIL TSIDGVPG.S DYINANYIDG YRKQ......
hPTPalpha_PTPRA       NKEKNRYVNI LPYDHSRVHL TPVEGVPD.S DYINASFING YQEK......
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hPEST_PTPN12          NVKKNRYKDI LPFDHSRVKL TLKTPSQD.S DYINANFIKG VYGP......
hPTP1B_PTPN1          NKNRNRYRDV SPFDHSRIKL HQ.....EDN DYINASLIKM EEAQ......
hTCPTP_PTPN2          NRNRNRYRDV SPYDHSRVKL QN.....AEN DYINASLVDI EEAQ......
hMEG2_PTPN9           NLEKNRYGDV PCLDQTRVKL TKRSGH.TQT DYINASFMDG YKQK......
hMEG1_PTPN4           NISKNRYRDI SPYDATRVIL KGN......E DYINANYINM EIPSSS....
hPTPH1_PTPN3          NLDKNRYKDV LPYDTTRVLL QGN......E DYINASYVNM EIPAAN....
hHDPTP_PTPN23         YSLKNRHQDV MPYDSNRVVL RSGK.....D DYINASCVEG LSPYCP....

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hHDPTP_PTPN23       ...PLVATQA PLPGTAADFW LMVHEQKVSV IVMLVSEAEM EKQKVARYFP

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hTCPTP_PTPN2        HFHYTTWPDF GVPESPASFL NFLFKVRESG SLNPDHG... ........PA
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hPEST_PTPN12        CIHCSAGCGR TGAICAIDYT WNLLKAGKIP EEFNVFNLIQ EMRTQRHSAV
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hTCPTP_PTPN2        VIHCSAGIGR SGTFSLVDTC LVLMEKGDD. ..INIKQVLL NMRKYRMGLI
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