Diverse Levels of Sequence Selectivity and Catalytic Efficiency of Protein-Tyrosine Phosphatases

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Supporting Information

Table S1. Peptide Library Screening Conditions

PTP	Peptide Library	[PTP] (nM)	PTP Reaction Time (min)
HePTP	II	500	10
	IV	2	10
PEST	I	1	15
	III	1	15
	V	1	15
PTPH1	II	50	15
	III	50 - 100	15
TCPTP	I	5	15
	III	10	15
	IV	5	15
PTPD1	III	500	60
PTPD2	III	800	60
	IV	78 - 500	30 - 60
PTPRB	III	5 - 10	15
	V	20	15
PTPRC	II	0.1	5.5
PTPRD	II	100	15
PTPRO	III	0.5 - 1	5 - 15
	V	2 - 10	15

Table S2. HePTP Substrates Selected From Library II (AXXXXXXXXpY)[a]

Intensely Colored		Medium Colored			Lightly Colored		
RRVFEDDD	SDDERNND	MSDADDEE	PSMVDTFD	DWDEIQEN	XFRFQDDE	DDGLWGTD	WWIDDSAA
IEPEFDDD	LELESNWD	VLAVDDED	SPVFDISD	INAEATEW	VEEEEAED	EWNSNWID	DEDGEQRF
SETNIDEE	TNSDVRTE	QQDEWEEE	AVSWERRD	SDEPQSDX	TEWDDQED	TSPTFWPE	DEDLDSWR
NDTDEFDD	REENFQWD	NLDILDDD	GFGVENAE	DAESVVEN	DATDEVDD	RFAWVWRD	EFDSDWAN
AEEQENED	ADDTRRWD	FWPNGDDE	XNVPDPIE	AADFMNDN	VGAVDLDD	TFRRQHRD	DNDWEGAS
VFGVESDE	IDETWSPE	GGVLDFDD	ADEETTLD	PNETIPEV	SEFEFLDD	DTALDDDP	EDGREHGS
TWVSDPDD	XXDTQVWD	ERGDQSDD	DNEDNMSE	GDWQSREW	PPTEWPDE	XXXFEEEF	FEIADSRI
QISGDIDE	INETTRSD	RASDQWED	DDADMSRE	STFTNSDP	DEDFSQDD	GVGNDDDF	DMAHEVHN
DEMDWQEE	RDHANHFE	PNSEPWED	WDLETANE	RSWEDDWF	XXEITNDE	EDDVIEDS	ADDENSTG
ADNEWFED	SAWSPFTD	TGFDWSDD	ENSERWSE	SLENEDWS	XXSFPWDD	ADNFIDDN	AQDDWWRQ
DWNDLNED	VWSNAVIE	AAWEWPDD	SVQDWRQE	DPWVDDQN	QNINWQED	VEAWNEDI	DEDVGWQN
EEDVWRDE	AWSDDDDS	PFNDWTEE	AGGEWISD	APDEWDST	IDEQEDME	VDTPTDDT	DDDGAWSV
GDEAQFDE	MDSIDDDT	PDSWSPDD	GEENWVNE	DPWDQDNI	FDIDSEFD	SSNFNEDF	GDDVNQWV
GDEWRVED	VDENNEEN	QFQRVRDD	TWENWTND	GFVDWEWA	DAFDSEIE	DVDFEQEP	DMENHPVG
DLEWGVDD	SRDIREEH	XXXXPTED	RQEGARQD	NNSWPEPT	PNSDSEWE	DETRDTEF	FVDMMRMS
TSDHGFED	TESWDFES	WFNDEDXE	WDSSSILD	XXNMSDGI	NASDSEWE	NEMPDWES	RVEAVQNR
EEWNPVDE	REEDQWDI	FDNLDDVD	AIWANFPE	FDEDDVHR	TSDMSDVD	XFAWDREI	DDSNSWAR
AEEWEDFE	RLFFSRDT	SNVWDERE	VQFPVRHE	EDFDDGRW	EDSWNEPE	SSDDNWDI	MEGNFQRL
SSNNDEAD	DWEDEDNI	SWVPEDTE	THRQACVD	NASDDQRR	FETSQEVD	XDSEWPEP	SQVMAWWP
QDDDWDNE	NDVWDDNL	RNDDLEWD	PTTHLRID	EVTSDATS	DGPFWEQE	RFLEQSDG	IGTLRNWQ
EVNDWDLD	SDQDMDWQ	AVEEFDND	RQDQDDEV	PTSAEIIW	XXNLSDWE	SDDMLLDS	PRTGQWVT
AFTDSDID	HRDAVDFV	GEFDADVE	DWQDFEEQ	NWAGEFGR	PEDDDVTD	GDTTTGDF	NVIGTRTX
RGESWDSD	SQDAIEIP	FDIDQEGD	DWQDFEEQ	ADEDAISR	SEEEENRE	XXFEDDWV	XXXISVTP
DDWPADSD	SEGEDMNN	DTLDPEWD	SSQEWDES	DDDSMWGQ	AMGEDQFD	SMDNDEQR	XXMRLNIT
IGDDESWD	AEFDPVWN	EAVDLDFD	VSNDNDEI	SDDTRIQS	NANEEWQE	NADGDDNL	XXAAALRR
AEFEDITE	NSPDQFRQ	ELFDPDQE	AADFMDDN	GMDGAPQR	XDEQDFPD	XEIMEDNF	HPSRPSQQ
NRSDEAPD	GEQSIVAI	NFSEMDMD	NEWNSEDW	PDTGSGHF	EDVGEGFE	DSNNDDAW	MQAPITAG
NEDSEWND	GFHSQFVP	XXVDSDSE	ADWQSDDL	VEALITGI	TEAVEPWD	DSNPDEIR	
RTDQDFFD	FAATTRMN	ADDGPDFD	FDMSVEDQ	EISRPHNN	LDVFDNLE	SVEDTDIP	
EFHSDRWD	NVASRPNR	SDEGSDQE	DANWRDES	XXXVTFAR	DSWTDQPD	DQQDFEFV	
ENGIEVQD		APDSWELD	TDDDDSDX	RRSPFGRS	DLDDIVGE	FPDGPDSP	
		EDFQMEPE	PNPDDSDW	FSRPNQNP	DEWDANVE	AHTPWDAP	
		AFDDDTLD	AGPDELDW	NRSPQVSA	XDADGFFE	EIDEENTW	
		VMAEDWSE	INLDEMES	IANVPTAA	XTMDWSVD	DNDEDSQX	
		DFDIDPTD	EATLEWDS		EEESFNNE	DADDDXXR	
		NMEPEWAE	RAGVDVDS		DDANAIWE	QLEDDSWR	

[a]M, Nle; X, amino acids whose identity could not be unambiguously determined.

Table S3. HePTP Substrates Selected From Library IV (AApYXXXXX)[a]

DEDQA	DDWVI	DWFEG	WDDTE	QWDSV
EDWEN	DDWVM	DWLDQ	VDDME	VSEVQ
DDWDV	DDVWW	DQWWD	WEDVW	VADQA
EDWIE	EDRRW	DGWID	WEDIQ	RHHDW
DDTPD	DDRRN	ERRWE	WDWDV	QAPWE
DDAQD	DWDQA	DQISD	FDTWD	SQIRE
EDFSW	EWEQA	DITPE	WESQV	WTSQN
DEWLF	EPDQV	DWTSV	WADDF	QWIFR
DEWQA	DWWED	DRQIA	QAEWD	NQQQV
DDWLS	DWVED	QDDDW	NWDWQ	SVTAP

[a]M, Nle

Table S4. PTP-PEST Substrates Selected From Libraries I and III (ASXXXXXpY)[a]

WEEDE	DWYQD	VVDDW	WEYDQ	FEDNW	GWDNM
MWDEE	DWWGD	FFEEL	WDYDS	WDDNI	LFDAP
WDYED	EKFID	SWDEI	WDYDA	WEDSM	LNDGG
EWWDD	ETHNE	SFDDL	EMWDW	WDDNP	EDWNY
PWFDE	TWGFD	WSEDI	DWSDF	WDENV	DEFAW
WGMDE	EDDDW	FTDDI	EAWDT	WEDVM	DDNWT
DWDLE	XEDEF	FNEEP	ELNEM	DYDWW	DDLFS
DWDLE	WDDEV	VSDDS	NWFDI	DYDWW	EDMWT
WFEYD	TDEEA	VSDDS	VFNDP	DMDWW	EDWNI
WFDID	EVDDW	MPEES	VFNDP	DWDWI	WDTNL
WDYFD	EWDDA	EEYDW	SWPDV	DWDSM	DLFSI
WENYE	GYDEW	EEWDW	FPGEI	EWDSA	DMWGI
WEIGD	WQDDF	DDNEF	QVSDV	DWDSM	EWQSI
WDGGD	FQDEF	EEMDF	EEDTV	SWDFS	WSTNS
ENWWD	WSEEW	QDWDY	NDDWW	NIDYS	TTNSS
DFFNE	WIDDF	GEWDF	MDDYW	GWDNM	MVRSS

[a]M, Nle; Y, F_2Y

Table S5. PTP-PEST Substrates Selected From Library V (AXXXXXpYXXXXX)[a]

DDWWQpYDALDF	PLDLDpYEFFWD	DSDDFpYAPDFW	QWDWFpYVDEPI
DAIDTpYDWQAP	WWDAFpYDNEMN	DVDMWpYSEVEP	PWEPVpYWDTDF
ELWWEpYDIQPP	PFDIWpYENWDD	DSWSDpYNIDDF	NFDSFpYSEFFN
EWQLQpYEELVF	GHKFEpYDDWWD	EFSFApYNDNDW	WIPDDpYIDNDM
DLWWSpYEEVGV	NFITDpYEFWDI	ADDEDpYGWAGI	SWQDMpYWEXXX
EQRTWpYDDWGP	TTQLIpYEEDWF	IEMDDpYMNFPW	WPWENpYGGDFM
PEDWLpYEIWPL	RAISFpYDDTWF	WEPDIpYGWTFE	MWQDIpYGWPFD
WDVDFpYEDMGQ	SQATFpYDDLWN	WDPEMpYSVFFE	INWSEpYLDLWE
RMDDMpYDMWPQ	SNLTGpYEDFFV	GWDEVpYTDWDP	NNQLWpYWEDDK
AFEFEpYEEPWM	EEDWIpYGWFEP	XADVDpYWEAEG	

[a]M, Nle

Table S6. PTPH1 Substrates Selected From Library II (AXXXXXXXXpY)[a]

Intensely Colored			Lightly Colored		
XXXKNKRR	XXXXXRQH	XXXXXSKL	HRISRRDK	XXKHRTSH	XXXXXLKQ
XRVPMRRR	RGRHHNVR	XSRKRRAT	FRRRIKER	RRWTPQTR	XHSRRRGV
RQRTHTRR	PFRKRAFR	XRRAKRQP	XXKRHDRM	XXLQRKRF	XXXRHRVM
XXKERTRR	XXXWSVQR	KRGPRRWT	ERRVHRPR	XXXXHKRF	XXXQRKFS
LRQRPPRR	XXXXNSVK	RQPPRKQT	XXXIHRHR	RNSGMRRA	XKRRTHTI
XRRNVAHR	KMRRRDRI	IRRQARQI	SPRQVRHR	XXXXWRRN	XXXKNRPT
KRIHHRVR	GRRPRGRW	RSRRRTPP	RRVVPNHR	XXXXTRKN	RANFRXXX
PPMHKRQR	SKRGRFRN	XRRVRGQF	XXXWQMHR	XXXXVKRT	XXXXKQNV
RPHTRRLR	XXKGRNRF	IGRNRQXX	XXRNRKFH	RHNRRAKN	VGRRSXXX
RTQFRRQK	KRTLQWHL	RRRKNNAF	XXXXKRTR	SHRLRLRS	RRQRXXXX
XXXWRKFR	XXXXFWHQ		XXXIKRGH	XARLNGRF	

[a]M, Nle

Table S7. PTPH1 Substrates Selected From Library III (ASXXXXXpY)[a]

Intensely Colored		Lightly Colored			
AYEED	EFEYW	YWEDE	EYQYD	WVDEW	YFGEG
WWPDE	DYEQW	FDYDE	DYIYE	WYEEN	AYVDN
WEDYD	EWEYQ	DYFED	DWVWE	PYEDV	AYADV
DWDYE	EWEYT	EYYEE	DYYAD	YDYEF	YQSDM
DEWFD	EWEWG	AWYDD	GSYFD	FDFDW	WEEYF
DFFFD	AYEQW	YIYDD	GAYFD	IDYDW	FDEYF
EFAYE	VWDSF	MPYED	AAYYD	GDWDW	FDETW
DWWAE	NDYSW	YYAED	YSSYE	YENDF	YEEGW
HAYTE		VYIDE	WSNYE	YDSDW	YDELF
TYGGD		DWEID	YSYSD	WDPEW	WDEAM
EYDDF		AYEWD	WNYSE	YEYEP	EFEYW
SWDEW		YSDYE	WAYNE	MEWDI	DPDYW
VWDEF		YIDYE	SQYID	EAYEF	DWDYV
DDYDW		YVEYE	VYTQD	DWQDF	FGDPW
WDTDW		VYDND	AYSID	DWIDF	VYDYS
YEVEW		EEWYD	AYGAE	DYVDF	YIEYS
WDFDI		YDFWD	ADDDW	DFWEN	DEYFF
FDYDI		NDYYE	FYEEF	EYWEV	EDSYW
DWYDN		GDWYD	FYDEF	EYWEI	YENTW
EYWEG		FEAYD	FFEDF	EAYEI	IDGYI
HPYDF		FDWND	TWDEF	DYGEI	DSNYW
SAYEM		YDWID	QYDEF	HPYDF	DWQYS
HPYDG		YEISD	TWEEW	AYADF	EYWSQ
YDDSW		DSYYE	VYDDW	WNYEL	DYWNQ
YEDNW		DSWFD	IWEEW	AAYDS	DAYTN
YEDIT		DAYWE	YHEDW	SAYEI	TYIFH

[a]M, Nle; Y, F_2Y

Table S8. TCPTP Substrates Selected From Library I (ASXXXXXpY)[a]

DHKWR	HRSRY	RRRIT	PRRMI	RIRTI	WRPGT
WSKAR	HRFKN	KRRSL	PKHLM	GFRXX	RSGAF
HRRKT	RRIHS	TRRQW	KSRGF	RRIIE	TAWQD
GHRRI	VRNRF	QRRFI	RARFT	RRWDI	QSSEW
RFRRQ	XKPKF	GRKFI	RGKFI	KKFFA	XXXGW
SPRKW	VRSRG	VRRFI	KFRTV	RKNFT	NYTWS
SFKRI	RFAKT	PKRWI	RFHXX	RRVFN	TAWST
GFRRA	RFAKT	FRRPT	RNRTT	RRIGQ	XXWGT
GFRRA	KKRYN	WKKQL	RSRAN	RRNAP	GFXXX
GFKRI	KHRFT	FRRIA	RGRNI	RRXXX	
XXHKI	HRRFI	PKRAS	RGRNI	FRSWN	

[a]M, Nle; Y, F_2Y

Table S9. TCPTP Substrates Selected From Library III (ASXXXXXpY)[a]

WEDEE	ITDQD	FSEDW	YDYDH	VWDWP	RNFFT
DWEED	ITESD	VTDDF	DQYDW	WWDTR	QGWTA
DWYDE	DEGTD	YYEEN	DAFDW	AIDTA	WGTSG
QYWDD	YDYFE	YWEEN	EIWDW	EEWSW	FMRPI
WGWDD	YEWYE	SWDDA	EWIDY	GEYSW	WGRVH
YWIEE	VDYWD	FDYDY	DWMDW	WDGNF	AITGN
YDEWD	YDSWD	WEYEF	EWWEI	FDYYS	STHTI
WFDYE	WDFIE	IDFEW	DIWEI	EYSWS	ATMRI
FYDWE	DWNFE	FDTDW	ITNDI	EWGYT	RIVRG
IYDYE	ETAWD	WDAEF	IISDA	DWFTG	
WNDYD	GFISD	WDWDN	DVDWY	DYFPM	
YMDWD	SFGGD	WDWDN	DWDWQ	EFFRP	
YYEID	FIITD	WDWDN	DYDFI	DPFTG	
YYEID	AVTGD	WDWDN	DWDRI	EFNTA	
YYEID	YFDEW	FDWES	DTETG	IQRNF	

[a]M, Nle; Y, F_2Y

Table S10. TCPTP Substrates Selected From Library IV (AApYXXXXX)[a]

Class I		Class II			
EVDEX	WDGXX	HRKRR	KRAQR	RVRNR	PTKIR
DWDWI	IDWNF	RRKGK	RRPAR	KLRXX	SIRXX
EWWEK	VDVWF	HRKVR	RRINV	KIRXX	SARXX
DWFDR	WWDEI	KKRFW	KRXXX	RIHXX	XXXRW
DWXVE	WWDQA	RRKGF	RHIXX	RLRXX	VMSXX
DWWFS	WWDXX	RRRPG	RFKRR	KIIRR	GIIXX
DWFXX	FIDXX	RRTRK	RQRKK	KAIRR	
WDEWI	NWEIW	HRNKR	RFKKA	KGARR	
WEIWD	FWWDK	KRARR	RIRKF	IRRKK	
WDWVT	YPYEI	HKIRR	RLRRI	MHKRR	
YEYXX	WTSYD	KRFAR	RMRKI	SRKWR	
WDYLV	TYWQD	RKVFR	RIKRV	NRRXX	
FEVWN	SFGSD	RRSGR	RVKTR	VRRXX	

[a]M, Nle; Y, F_2Y

Table S11. PTPD2 Substrates Selected From Library III (ASXXXXXpY)[a]

WEWDD	WQEDI	DWYDV	YYDFN	SDFQF	DYHIT
WFADD	WHEDL	DYYDI	FFDYT	WDSGN	EIITG
XIYDE	HYDDI	EHYEI	WSENV	XDAYS	EGYTI
SWDFD	IGEEI	QTWDS	IYDYN	PDNTM	DYYGI
FWDQE	DEWES	SYPDI	YNDYI	VDYNI	ISNGW
EEHYE	LEYDF	XXLDM	GSELA	VDYLI	WATFR
EWFAD	SDFEI	SEEYW	DENFW	EAWTA	SNYQI
FTFGD	YEYES	WEEAI	DEWYF	ETWMI	XTGNA
WWISE	XELDT	XEDSI	EEWWG	EWYTT	
TSQGD	YEYDI	XFDSW	DEWTM	DFIGS	
AWEEA	ETWDI	VTDTW	DDFYY	EFTSL	
XWEEI	DWYDV	FVDFS	DEYTI	DFHTA	

[a]M, Nle; Y, F_2Y

Table S12. PTPD2 Substrates Selected From Library IV (AApYXXXXX)[a]

Class I			Class II		
EDEWF	WDIEI	YLEDY	RHKRF	RTHRR	GHMRR
DDWEY	VEYEY	YGDDY	RRRRS	KIHRR	SRVXX
DDQWW	ADYEY	YADWE	RKRSH	RVRRN	LRQXX
EFDWW	YDVFE	YTEFF	KRKMR	RQFKR	SAKRR
DSEYF	SDWFD	AWEHN	RRRPR	RNSHR	
EWNDY	IDWWD	TVDYY	RRKIF	RMIRR	
EYHXX	YDFFR	WQFDD	RRRXX	RIXXX	
EVWRY	YDYFA	YSWEE	RRRXX	QRRHR	
WDEEI	YDYTT	WSSEY	RKFKH	SRHRK	
WEEYE	YEWSM	WVNEY	RRVKR	GKRKR	
FDDYF	YEFIH	WITDH	RRFRT	XRKRW	
FDDNW	YDNFS	TGYEW	RRMRS	MRRKN	
WEWDE	TEWYY	MRNEK	RRASR	IRRRI	
YDFEY	WYDEE	YSHWD	RRINR	IRRSR	
WDMEF	YYEEF	AWQFD	RRTXX	LRRAR	
YDVDF	WWDEQ	GRXXD	RRNXX	MRRFV	
WDTDS	YWEEQ	IWNHW	RRXXX	IRNRR	

[a]M, Nle; Y, F_2Y

Table S13. PTPRB Substrates Selected From Library III (ASXXXXXpY)[a]

FWEED	DWFFD	WDYEA	EFDLF	NHDRI	DPWXX
FYFED	DWPWD	SDWEM	DWELF	DDYWT	FGWGW
WLYED	HSLFD	DWVEW	EWEWN	WESSW	RYLFX
FIWDE	WXXPD	DWYDI	DWDWG	WDNVF	INYVS
WWQDD	TSGTD	LGYDW	GYDFY	WDNVF	SNFSA
YWIED	YYDEF	TSWDA	YWDTT	LEFWP	MPWNI
WIHDD	WQEEW	FGIDV	YWDTM	PDFTG	ITFXX
WEEWD	WIDDF	SSIES	WFDXX	WDIXX	FRSNV
WYDYD	YWEES	YDDFW	FYDPI	WDLXX	WNTXX
WWDYD	FWDEM	FEDTW	SWDIS	NEVTA	VNTNS
FFDFD	WSDDS	WEDSW	GFDTA	SDSVG	NIIST
GMEWD	ITDDI	FDDNF	GWDXX	QDXXI	HPITS
WMEID	ITDDI	FDDIW	FIDGM	DYYPF	ISNIS
WDGFD	NEYDW	IEDXX	ITDNA	EMWYP	SHQVX
WEWID	IDWEW	DWDFY	ASEAV	DWWPQ	

[a]M, Nle; Y, F_2Y

Table S14. PTPRB Substrates Selected From Library V (AXXXXXpYXXXXX)[a]

XXKWMpYKRNVK	AMAPIpYGRFKQ	RRNFTpYRGGIM	QSNRFpYAKRRI
VPNAMpYRMKKR	ATNNLpYGRMIR	KPNITpYARRGR	NPEADpYMKRPR
QLTEMpYRGKRM	MHKDMpYAGKRF	RFFPNpYARLKR	XXXXXpYGKRFK
GTQGMpYRQRWD	ASPNMpYSNKPK	RREGNpYXXKWQ	XXXXXpYGRKWP
QRPTMpYRTRXX	IKRRVpYGMRGK	RGKSTpYVFNQR	XXXXXpYXKKGM
KGNKApYRGARF	IPPGMpYGFRGQ	MRXXXpYRHRRI	RKMRPpYIRKQG
FSADVpYRXXXX	ASSHVpYNAVRR	LXXXXpYRVHRS	NSXXXpYAQPRR
MPDNIpYTRIRR	RPFSIpYGXXXX	RFATDpYRQRPK	RRQPXpYXXXXX
XXXQVpYMRPRT	KLRGSpYRQTHF	XXXXXpYRIKLK	

[a]M, Nle

Table S15. PTPRC Substrates Selected From Library II (AXXXXXXXXpY)[a]

Intensely Colored		Lightly Colored			
IEIDWDDE	WWDNGIDF	DDIVWDED	QFWEWGGD	DEIEVNDW	XIEDEIWG
WSWAMDDE	SWDNVWEG	EHPFWDDD	FEDFASFE	ADAEFWDS	DEPDEGWV
IPWDDWEE	DHGNQSDM	ELFFSDEE	DMDIWQPE	DLQDWFDF	SETDEQIW
FWLNDWDE	AIQWGNDW	ITMAWDDE	TNEIWQWD	XTSDIWDF	IESEDWQL
WVEDGWDD	NGTSWWDQ	WIDVERDE	QSDPISWE	VSSEWWDN	EWWEDTFV
TWWDWWDE	XNNWAWDA	EDMWDMEE	XWDWIVND	VWNEPWDA	DVGDDVWP
WLDWLQDD	TNEEDEFW	TTIWDWEE	LDQSWVWE	NEDPWNEV	NVQDEWPW
MDNIFWED	WGIDDDFN	EDWEPIED	WDSTTWVE	ISEFANEW	HWADDNQF
AEQMWWED	DWFMDDSW	NNWDSFEE	DWPTMAWD	TWDQTIDW	PTADDWSN
EWWINSDE	DSWWDDAI	XPDNWFDD	DAFSWSPD	GWDVMWDA	DIDWDXXX
NSQWWPEE	VEWDWDWS	AMEPWLDE	IVNEDEDL	DETWGWDI	NVEWDFGQ
QVWTDDWD	AIDPWDPW	AENWMWEE	IFTIDDEF	XDPWTSDW	TVDMDWNG
TQWDSEWE	WNDLLESW	IKSSAWDE	WTTNDEEF	GDPMLNDW	EDNWDGFM
WEAGPDAD	DMWSQDWV	FTFPWNED	XWWVDEEG	NDFAWFDP	NDQLDWLL
TDNWDWND	EWEEDQWA	SPWWTVED	WVEDVEEA	WDNINWET	FEWAEPIT
WDEDLVWD	DEWEDWII	ISDWDEID	FDIDSEDF	EVMNWWDV	PEWNDMMA
VFNDWWTD	IDFDDWWW	WVWDFDME	PNIDGEDW	XDDMDEWA	DEDDFNSW
MDDTWWPD	GDGDEWWT	SVPESDVD	VWWETDEG	DNENDDSW	DEEDFFWT
ESEWWRSE	DTWDDLWA	SDFVMDQE	VEEWFDEI	VEFRDEIF	DSEDQWNN
DDMWSNWD	WNNEDWTW	DWSWSDNE	SEPWPDEW	EFITDDTW	DIDEWLMQ
XDWDDEEI	DDDLDSWQ	LWWGTDFD	SDGWNDES	SWGMDEMW	SMDDWLGI
LWSTEDEW	DVDWDTMF	GFSSWDSD	WDSQNEEV	XFDEGEWS	XETESFGW
WDFDVEDV	XEPWEQWW	GWTWLDQD	WGWTTEDV	VWEDSEVL	FDNDWNWQ
DWWDSEDM	SEWNDWNN	ITWRSDQD	XWSITDEA	WIWDSERW	XEWDFWLN
EDWWAEDS	XEEEWWWG	IDDDDPWD	AFTDDWDQ	IQQEFDWA	WDWDPSIL
DAQIWDEW	DMDDNWTI	QEHDDWWE	XWQEDWDS	WGLDTDFN	ETNDNAWW
WQPWTEDT	SMDDNWWQ	MWEAESGE	WDVQDPDW	WINEWDQN	ELWEAATW
DFEPENEW	WNDDWLLQ	GWPAEWAE	TESWEWDT	IEEWKESV	XVADWFWW
EQMWDWDT	XDDWNWTN	SVVSEWND	DWNIDNEF	WEETLEPT	LWLDSNPR
XNFVDWDW	ESDVLWQW	GDDESVWE	GVGIEWDW	DFEWFDNQ	TDEWLNQF
MVWPEWDV	WNDNPSIW	FSEESQWE	XFITDAEW	EIENWEAM	MDDFQWTP
XXXDWPDW	DDWMVWPN	EFNEWITD	PGSNDWDN	NTEWWDTV	DQEMWNSM
WWADSMDQ		XINDFVWD	WWGFDVES	DNWQGDAW	XNDWVSNW
WDDSFSDV		WVIDSPWE	MEDELMDL	EWGANDLW	

[a]M, Nle

Table S16. PTPRD Substrates Selected From Library II (AXXXXXXXXpY)[a]

Class I				Class II	
Intensely Colored		Lightly Colored			
XDWIDWEN	AWFDETQW	VWDLMWEE	AIEKWWDM	XKRWWKNN	ARNRKRGN
NDFFLGDW	WLDPDATW	EWDWMEID	AWTEMDRW	PKSRFRQQ	SKKRQRNI
DFVTWVDF	SGTDSWSW	WDWGAAFD	MTFWSDSI	IKRWPTRL	QVGRRXXX
DSWWXXDW		AFMRKKMD	PADWDPMM	FRSHQQRR	AARKKRLI
TWLETDLF		XXIDFKDI	PDDFNWNF	RRLRSRVN	

[a]M, Nle

Table S17. PTPRO Substrates Selected From Library III (ASXXXXXpY)[a]

FFEWD	LWEDF	ESWDM	EPDWI	DDFWI	EHVTA
WNDFD	VWDDF	QMWDF	ETEKV	EETWM	TFQTW
NIDFD	FMDEF	WSSDF	WNDMF	DDAXX	ITAVF
NQDVE	WYEDV	WATDW	ITDNF	WEPYF	GTGYL
EDWWD	YWDDI	AMGDF	GSEGF	NDSWY	GTGYI
EEWWD	TWDDL	NYWEP	TWEWT	NEWWT	XXIWP
FEWYD	QWDDI	IWYDI	TWEFS	WDWAV	NWFAI
FDLWE	WIEDT	PFFDI	FFDNS	SDFHI	PWWXX
FDIWE	WEYEF	WAFES	FFEHT	WEHSP	HTWQI
FEWGD	VEWDW	FMWDV	YFEQV	SDINH	ISYGI
MEWTD	WDMDF	SGWDM	WFDXX	SEHTI	MWGTA
WDVLD	WEIDW	FFTDT	PFDTI	EFWFF	SWTXX
DWWND	IDMDW	DDEFW	VWDTA	DPFYY	WNSNR
EWYSD	WEWDI	DDDWF	FIDTV	EWTNF	FGNTA
EWNSE	WEWDI	YDDFW	WIDSI	DFWWS	WNSXX
FNIWD	IEWDM	FDDYM	FNDXX	ESWWN	WSGXX
WYYND	ESWDW	FDDHS	FPEXX	DFIWS	TITTM
FVWPD	DVWEF	WDDTM	IGEAS	DFAWS	NPQTI
ATWGE	EIWDW	WDEXX	IGETG	DTIWN	GATTA
TWVND	EWPEW	EWEFF	MVETA	ETWGT	GITSM
IWXXD	DWHEF	EFDWF	DENFW	DAWTA	HTTGG
WIHSD	EWWES	DFEWN	EEPFW	ETWGM	KNHAP
NVIGD	DWYES	EWEYI	DEWNF	DFASP	ASGGM
WEDEF	EWWEI	EWEFI	DDWMF	DFHXX	TKGAI
WEDEF	DWYDV	EWDWG	DEWWA	DIVNT	

[a]M, Nle; Y, F_2Y

Table S18. PTPRO Substrates Selected From Library V (AXXXXXpYXXXXX)[a]

Class I			Class II
FFEQApYFEDNH	NNQXXpYMFDEE	TQXXXpYDMSWE	RQFRKpYGGRKE
GLNFGpYWDDFG	EWFDSpYAWDIS	XXXXTpYEFGQD	RPTNFpYRQPQR
XXXXXpYWDSEE	XXXXXpYMWLDD	DFWWDpYEVWPS	DQWDEpYSPWFS
PIXXDpYWDVDT	PFEDFpYMPWWG	EXXWDpYEPMWN	RGIQDpYGQFFR
FENEApYWEVDF	FSQEIpYVSWAN	AWQDMpYDWQPF	XXXXXpYGIQTW
IENDMpYFDSEL	AWGNFpYEEEWS	AISDTpYEAWWN	LTXXXpYGLKTW
FQMFDpYFEPPE	TNXXXpYEDWEI	ASVDSpYDFASF	DSNSIpYQVRKR
NEEVFpYWELVE	SEXXEpYDEIFD	TTSNWpYDMSPV	VNGXXpYKANKK
XEAATpYWELFD	TNPAWpYEDWLE	XXWDMpYQEDGK	RQTXXpYGRPRS
SDWGIpYWDQQE	XXXXXpYEDPND	IAFFEpYQDMEW	RGNALpYRSHKM
GMXXDpYWENMW	DAWWDpYEDLFI	VAWDNpYSEWDV	RFKTSpYQSRQR
ARNDFpYWDMSV	FWSNApYDDFWQ	XXXDKpYSEMEW	RRSAVpYRGGHK
VSWWWpYWENPI	SWSVPpYDDWPI	WGNXXpYNDWDV	XXXXXpYGKKRM
FPFIPpYWNDED	AELFPpYEEWIW	VDFWGpYQWEDD	SIGXXpYRRFKQ
SGPXXpYWWDEV	GEWMFpYEEGFQ	EWNTFpYSWDES	SNRRApYGRLMK
TPSVQpYWSEEM	EPWTWpYEDWGM	QWSEDpYTFEWE	RRFASpYNSKRM
TWDTNpYWVEVD	TWQAVpYDDAWF	XXXXXpYQPEQE	TRKGMpYGSRAK
XXXRApYFMEFE	XXXXXpYDWEDE	PFTDWpYTFETT	XXXXXpYXKSAK
APFFDpYWIEPP	WLNSPpYDWDEI	GRDMFpYTFFEE	GMXXXpYGRVRW
LITDIpYWSDFS	SXXXPpYENEDT	NNRNIpYSYSED	XXTVPpYGRKIK
EIDPWpYWSEGF	DADVWpYDFDLE	LWEDVpYSWGDH	LQRGPpYKRGRR
IQDPTpYFWEPT	DPIEIpYEIDWF	XDDWFpYSTLEN	VKSGQpYNFRRI
TQFNDpYWFMEE	VXXXXpYDWIEE	SXXXXpYTPWEQ	KADGIpYTRRRF
FPIEWpYWPGED	WNVFDpYEFAET	XXXXXpYSWAEN	KHRGVpYAHRKF
MEDLFpYFGSEW	PEFDWpYDNAEF	ISGEWpYNNWNE	DERQApYVRGRK
IXXXXpYWGFEV	PADGFpYDSSDW	WGDDFpYSIPPW	ARNFRpYATGWG
PSDWDpYFNFLD	EWAQApYDWFEN	TFDEMpYTTTPN	RKTGApYGFKSR
QTEEFpYFWNPE	XXXXXpYDGSDF	QHDXXpYGDWKD	ATKQApYRITRR
VEMDPpYFWQWD	DVWNWpYDRFDQ	NRMPQpYGEEFMW	NXXXXpYGWKPR
ESXXXpYWQPWD	XXXXXpYDSWEW	IFDVDpYGREFW	XXXXXpYRKAQR
QGXXXpYWMPWE	IRXXXpYEVFDW	LSWQDpYGGWFD	XXXXXpYRRKKW
FINEFpYMDLDW	NVFASpYDWPWD	FWDSTpYGPRVV	IRLRRpYGQKMK
IDWVApYAEWMD	IEWIFpYDQWAD	WLDDEpYRVWDP	XXRMMpYRQAKR
QDFESpYMDWSS	WEWSVpYDVNWD	IRNXXpYRDDDF	RVHAFpYGKKVW
XXXXXpYADTTA	SWXXXpYDPMAD		

[a]M, Nle

Table S19. Kinetic Constants of PTPs toward pNPP Substrate

PTP	pNPP		
	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1}s^{-1}$)
PEST	6.7 ± 0.2	2800 ± 300	2400
PTP1B	20 ± 1	2700 ± 200	7300
PTPRC	77 ± 4	9900 ± 900	7800
PTPRO	99 ± 4	2800 ± 300	35000
PTPRB	56 ± 1	350 ± 30	160000
TCPTP	6.1 ± 0.1	790 ± 50	7700
SHP-1	14 ± 1	6000 ± 250	2300
PTPH1	1.3 ± 0.1	1100 ± 200	1100
SHP-2	12 ± 1	9200 ± 1500	1300
PTPD2	0.036 ± 0.001	2100 ± 100	17
PTPRA	2.5 ± 0.1	4800 ± 400	530
HePTP	3.2 ± 0.1	9600 ± 600	340
PTPRD	0.29 ± 0.01	2400 ± 300	120
PTPD1	0.014 ± 0.001	5500 ± 200	2.5

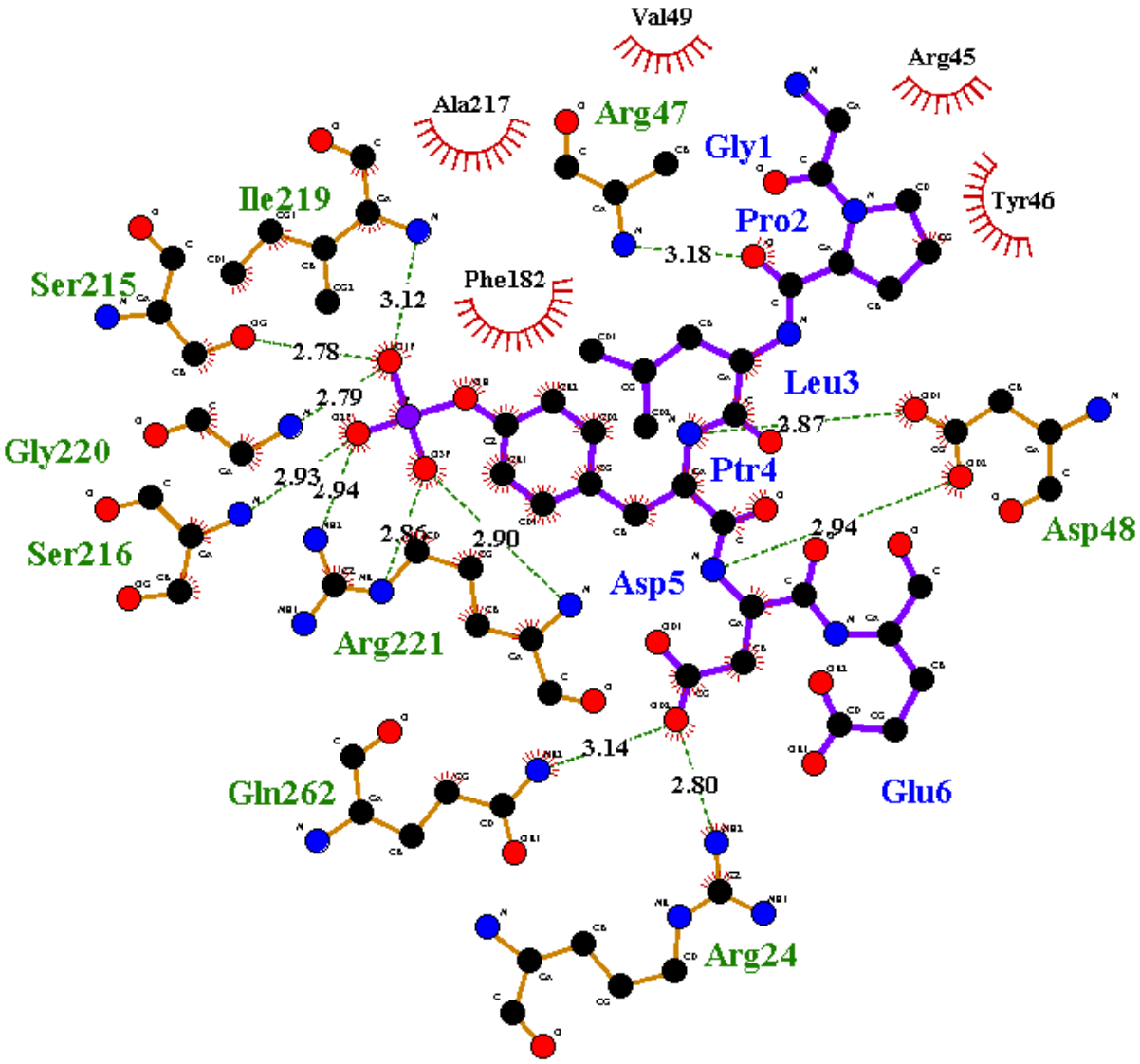

Figure S1. 2-D representation of the interactions between PTP1B and the Nephrin peptide AWGPLpY[1193]DEVQM. Direct hydrogen bonds and electrostatic interactions are shown as green dashed lines. The atoms are colored as: black, carbon; blue, nitrogen; red, oxygen; and purple, phosphorus. Distances shown are in angstroms. Graph generated by LIGPLOT.

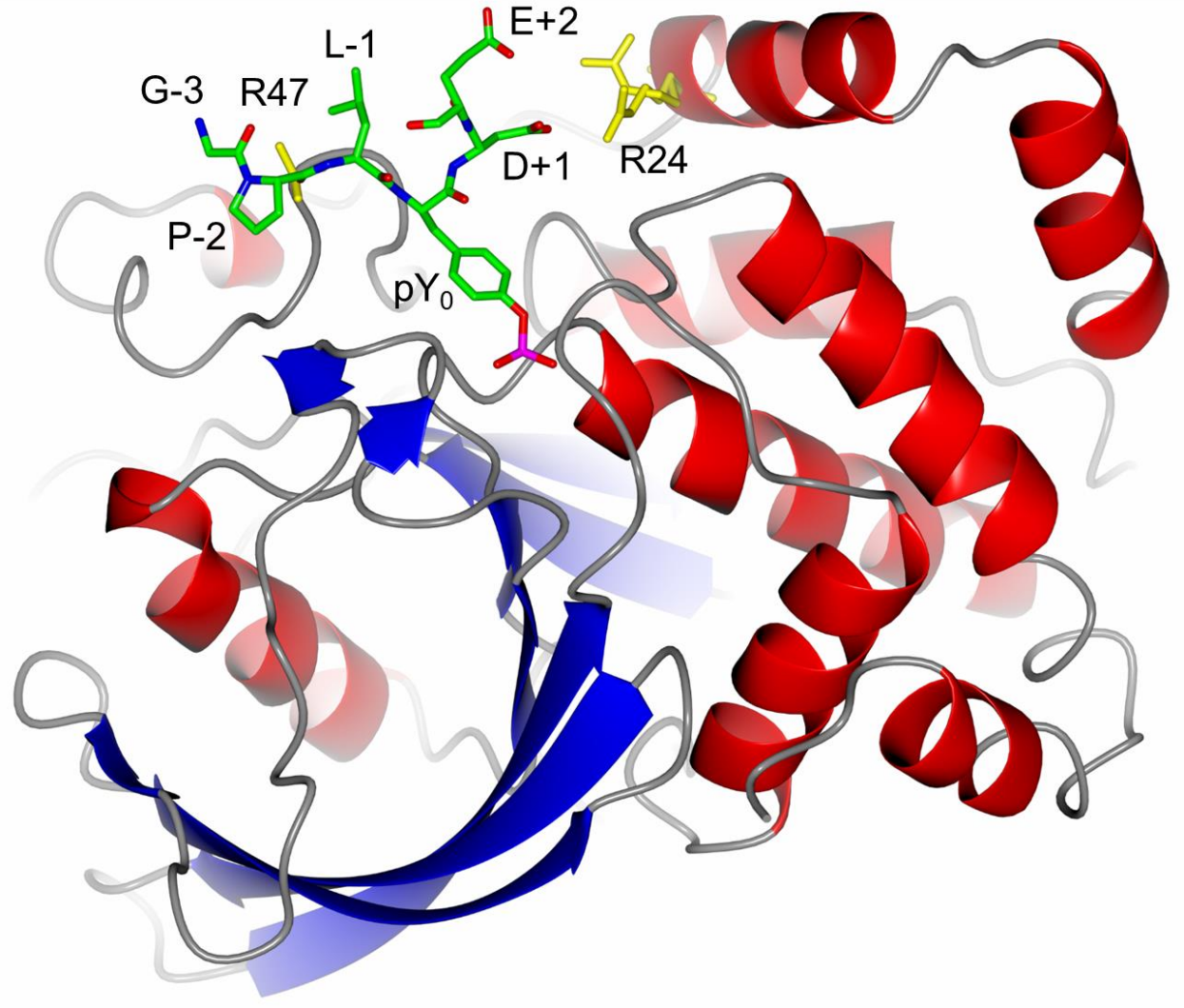

Figure S2. Co-crystal structure of PTP1B-nephrin pY^{1193} peptide showing the two alternative conformations of Arg24 side chain. The side chains of Arg24 and Arg47 are shown as yellow sticks.

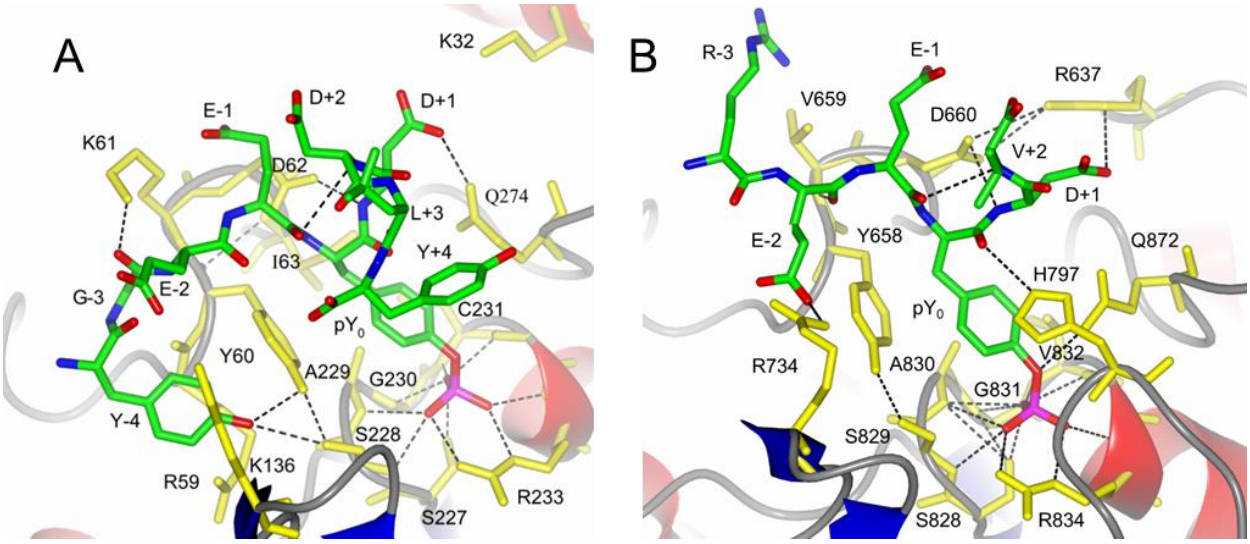

Figure S3. Co-crystal structures of PTP LYP (A) and CD45 (B) bound with pY peptides. For (A), the sequence of the pY peptide bound to LYP is YGEEpYDDLY (from pdb: 3OLR[1]). For (B), the sequence of the pY peptide resolved is REEpYDV (from pdb: 1YGR[2]). The peptide (green) and interacting residues (yellow) are depicted in stick representation. Hydrogen bond interactions are shown as black dashed lines.

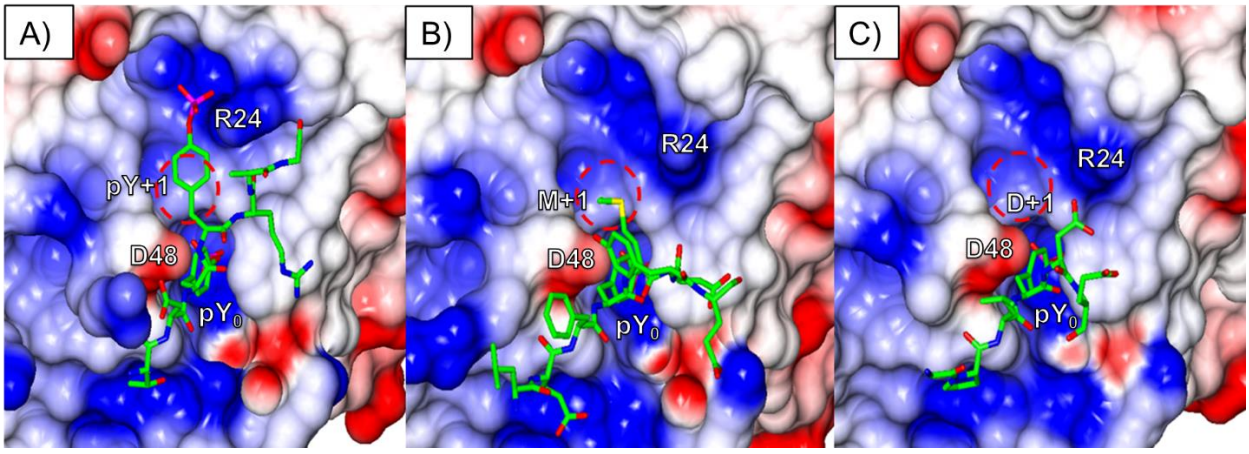

Figure S4. Interactions of different pY+1 residues with the amphipathic pocket on PTP1B. (A) PTP1B-insulin receptor peptide complex, where pY is the pY+1 residue (generated from pdb: 1G1H).[3] (B) PTP1B-consensus peptide complex, where the pY+1 residue is a Met (generated from pdb: 1EEO).[4] (C) PTP1B-nephrin peptide complex, where the pY+1 residue is an Asp. The broken red circle depicts the hydrophobic region of the amphipathic binding pocket.

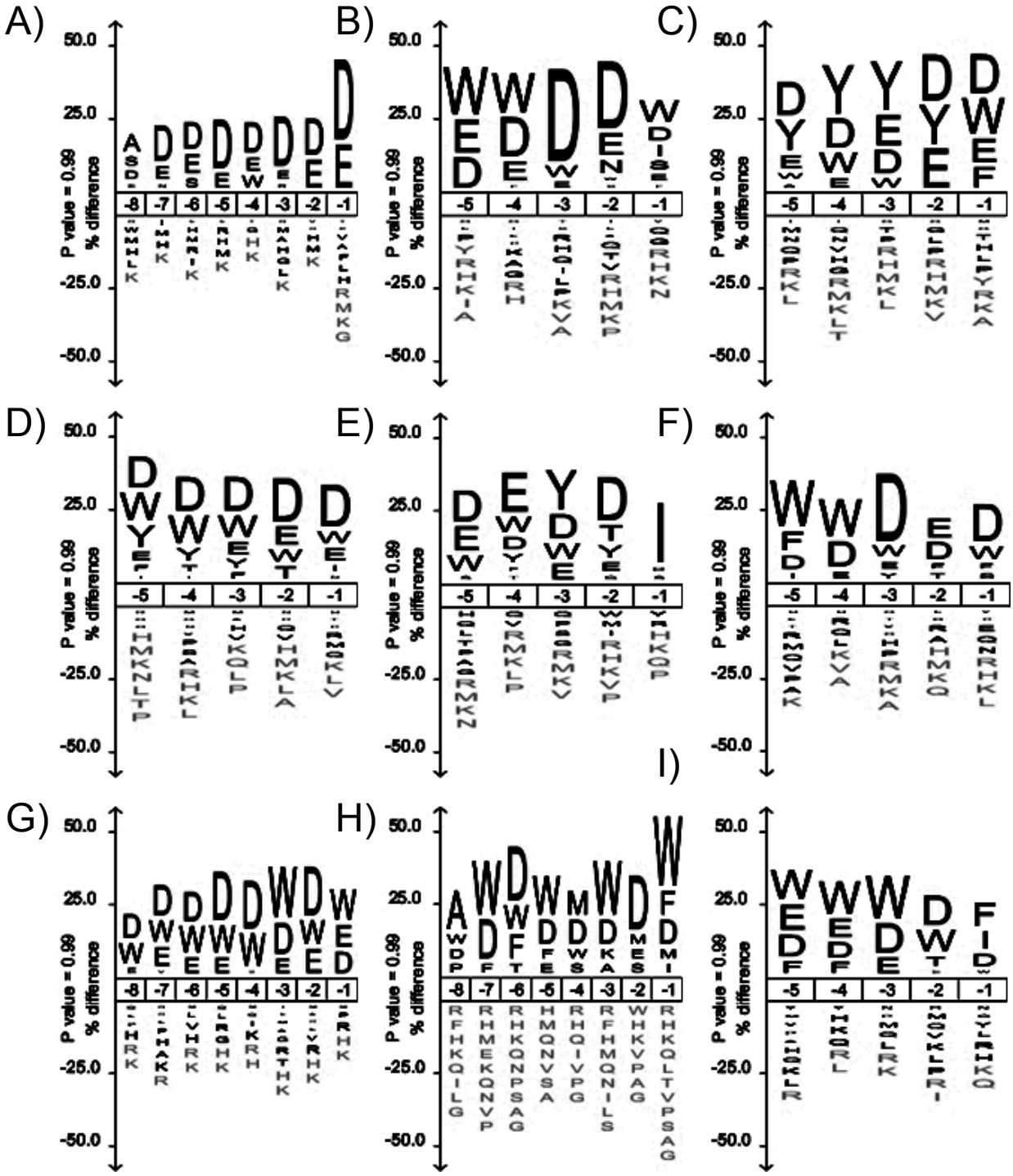

Figure S5. Icelogo plots showing the sequence selectivity of HePTP (A), PTP-PEST (B), PTPH1 (C), TCPTP (D), PTPD2 (E), PTPRB (F), PTPRC (G), PTPRD/Class I (H), and PTPRO (I) on the N-terminal side of pY. The y axis represents the percent difference in frequency of appearance between the selected peptides and the initial peptide library at a given position (pY-8 to pY-1, x axis). M, Nle; Y, F_2Y.

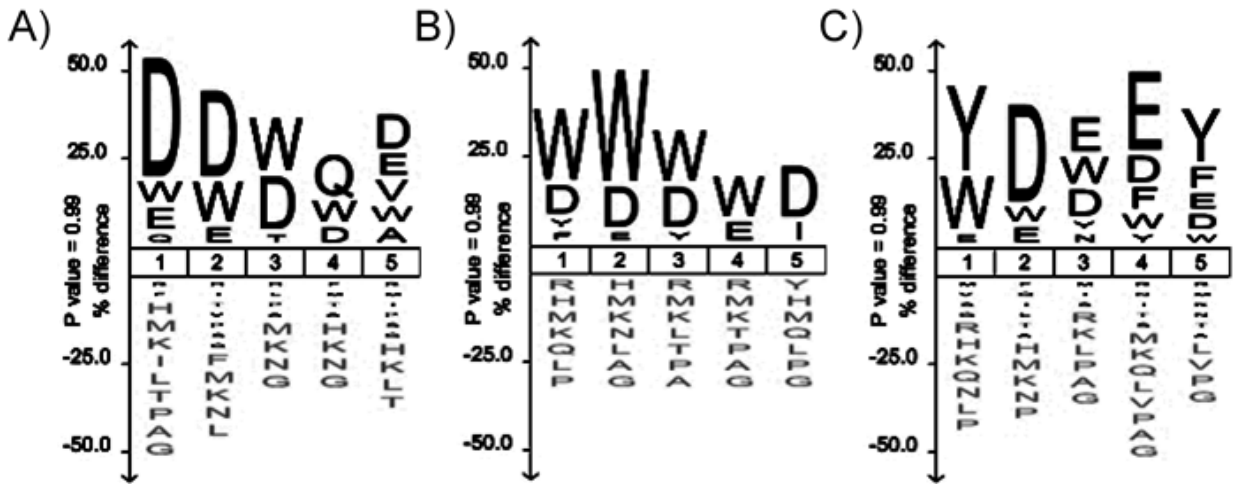

Figure S6. Icelogo plots showing the C-terminal selectivity of HePTP (A), TCPTP/Class I (B), and PTPD2/Class I (C) as determined by screening against Library IV. The y axis represents the percent difference between the selected peptides and naïve frequency of the peptide library at a given position within the peptide (pY+1 to pY+5, x axis). M, Nle; Y, F_2Y.

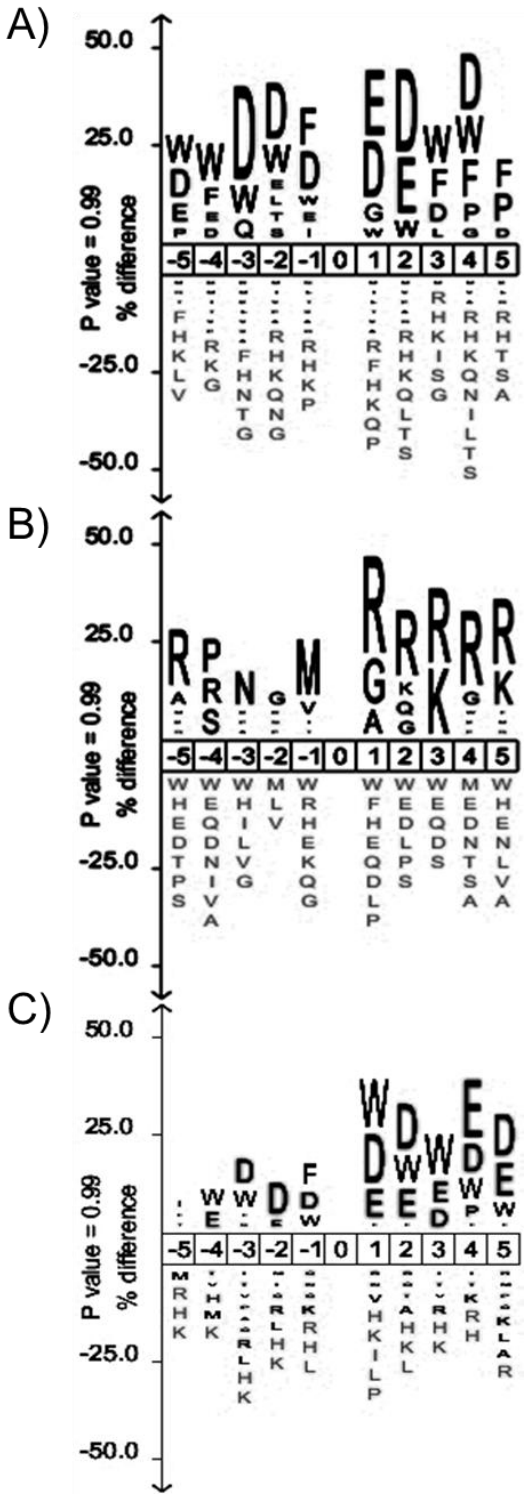

Figure S7. Icelogo plots showing the sequence selectivity of PTP-PEST (A), PTPRB (B), and PTPRO/Class I (C) as determined by screening against library V. The y axis represents the percent difference between the selected peptides and naïve frequency of the peptide library at a given position within the peptide (pY-5 to pY+5, x axis). M, Nle; Y, F_2Y.

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