

# Specificity Profiling of Protein Phosphatases toward Phosphoseryl and Phosphothreonyl Peptides

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Supporting Information

Table S1. Preferred VH1 Substrates Selected from Library II (XXXXXXZAA) at 100 nM VH1<sup>a</sup>

Intensely Colored		Lightly Colored			
Class I		Class I		Class II	
DDFEAt	AIAAFt	EEYYt	IIFQAt	DFEYYt	YEGUYt
DEFYgt	QIAFAt	EEFGPt	EYIGgt	EYDFLt	IEGAGt
DDYMHt	VIMPnt	YDFAAt	FGIAMt	EFDQMt	EYNNnt
NEFYpt	EGPENT	IDYUNt	IQUIIt	EFQGGt	EYNNAt
GEFYpt	PNUFnt	NEFVNt	EDVGft	GDNQMt	INQMLt
NEFMAt	EEYVNt	NDFGpt	EUUYnt	EENFAt	
*EFYUGt	EFVENT	IDYMGt	EIUInt	EFQQDt	
EFFAGt		ADFMAt	QPUUFt	EUGDMt	
EUYUGt	Class II	DIYYAt	HUUQQt	YHDFIt	
EVFIQt	DFQUAt	DFYHGt	KEFEMt	RVDDLt	
VVFUNt	ELQFQt	EAYUNt	NDFFYt	EFDDGt	
QQYVNt	FVDYFt	EMYMNt	EYFEAt	VUEUHt	
VQYVGt	NYEPPt	NFYEQt	EEYFQt	HYEEGt	
DFVVGt	DFEUHt	MNFENt		FNENNt	
EQIMNt	MNEYFt	ILYUQt		MQEQQt	

<sup>a</sup>M, Nle; U, Abu; t, pT; \*, sequences selected for kinetic analysis.

Table S2. Preferred VH1 Substrates Selected from Library II (XXXXXXZAA) at 1 μM VH1<sup>a</sup>

Class I			Class II		
DYFVDt	EEYFAt	EYMNGt	GNDEFt	EDEQFt	HDDDNt
EEFYgt	DFFDAt	DUIEDt	NEEVGt	QLEYAt	ELQGNt
EEYYIt	ENFENt	AGMEQt	GFEYFt	MDDAht	EYNUYt
UEAYDt	DAYDDt	YDLMHt	LIQMft	AEEFAt	DFHNYt
EUIYNt	EFYYEt	QEMAFt	NIQAFt	VYEDYt	NUHNI <sub>s</sub>
ADIEFt	EEYIGt		PFQEAt	IUEAEt	EYHIDt
EEVLYt	AQYAGt		DYGDYt	HEEFYt	HLGYHt
LGVNLt	EFVENT		DIGVNt	VEEEYt	VNGUFt
VVUFEt	NDMYNt		QQGYDt	YEEEDt	QUGEFt
QQUYGt	DDVIHt		UFHDGt	GNEYNt	EUGDMt
	AFVDGt			DYDHI <sub>t</sub>	EHGFFt
	ELLYDt			EIEYGt	PDGINt
	KDVGMt			PNEYIt	

<sup>a</sup>M, Nle; U, Abu; t, pT; s, pS.

Table S3. Preferred VH1 Substrates Selected from Libraries Ia and Ib (ZXXXXXAA)<sup>a</sup>

Library Ia				Library Ib	
sDIIIFI	sFEFFP	sEGFFI	sFMIEN	*tDFIQF	tIEFIN
sFDIFF	sLEFAF	sIEDIW	sMFADI	tDIQFI	tLFEDI
sLDFIW	sIDIII	sFEFDI	sLGFDF	*tFDIQF	tFFEHF
sIEIFI	sAFDFT	sFEIDI	sMIFEN	tFIDIE	*tFSDFI
sVDIFV	sMFDIV	sIEVQF	sVLFDP	tMFEFE	tISDFI
sLDIIF	sVVDFP	sMEIIQ	sIVIEI	tLMEFF	tILEAI
sVIIDF	sAFVEF	sIEIIG	sFFGFD	tLMEII	tSFIDE
sMLIDI	sIFIEN	sLDVII	sGFIFD	tFFTID	*tFIADF
sLIIDI	sMLFDF	sIFEDS	sVFIPD	tDEFFI	tMFIDI
sDFEFF	sMFSED	sFSDFI	sLFIGD	tDLFEI	tLMFEP
sEFTEF	sFIFSE	sFMDVV	sLIFTD	tENFII	tVTIDF
sDMFEN	sATFFD	sLFEVN	sAGIFE	tENMWF	tFFVQE
sEIIIFD	sIEWIN	sMAEAF		tDPLFF	tFMFIE
sEFTFQ	sEEFAI	sLMDVT		tDVITF	*tFLIVE
sEIFGF	sDFDFN	sFSFDI		tMDDFI	tGFFIE
sEMFVN	sELIDI	sFLAEF		tMEIDI	tVFSFD
sDIIAF	sEVWID	sFMIDN		tIDFWI	tRIIFD

<sup>a</sup>M, Nle; ; t, pT; s, pS; \*, sequence selected for kinetic analysis.

Table S4. Preferred VH1 Substrates Selected from Library III (ASXXXXXpYAA)<sup>a</sup>

*AQDFDy	ADLYSy	FSNEEy	LIGDSy	FFNGDy	PFQEGy
SNDFDy	LEPFSy	IFEENy	ELWSNy	WANGEy	FEIDPy
TADFdy	LDMFPy	RFDENy	IEDSNy	FEAGNy	ELLDPy
SEEWey	IDAFGy	FPEDNy	FAFNEy	IHMANY	AMDSMy
VDMFEy	IGDMDy	MIEDTy	IDFNdy	QIDDFy	PEDSMY
ELNFEy	ADWLEy	AIDESy	MNYSEy	*ADTDFy	DYAQAy
AYPFEy	FDSADy	EFFESy	FFNNEy	DDLEFy	FTESPy
AQYFDy	ELWADy	GFFENy	EYQSDy	MNDDIy	FEGNGy
DDDFQy	FKWDDy	LGWESy	FPGNDy	DAPDIy	EFGGMy
DNDFSy	SLFDEy	*AFGDSy	FEHSEy	ESWDLy	WEDGAy
LNDFQy	AFAEEy	NYPDSy	FESSDy	MVFDAy	LDDPMY
DDTFQy	FVTDDy	PFIDSy	VMESey	AFEDPy	EPEMWY
*EDAFSy	YLNDdy	FPIDSy	LDDGEy	ISDDGy	EDALYy

<sup>a</sup>M, Nle; Y, F<sub>2</sub>Y; y, pY; \*, sequences selected for kinetic analysis.

Table S5. Preferred VH1 Substrates Selected from Library IV (Alloc-AApYXXXXX)<sup>a</sup>

yIVWEE	yDMWLA	yWEYQI	ySDWFM	yLFDFT	yNRIDF
ySAYEH	yEIFIS	yDFWQT	yENWWP	yWIDVY	yDIMAF
yFVYDY	yYPPAW	yQPYTT	yDRWFE	yWNEIF	yWPNDG
yHMYES	yFDWIS	yEVWNR	yGPYHE	yDWMVP	yFEQYW
yFQWES	yHDWMW	yHMWTE	*yQWDSF	yDFAYQ	yFEQWA
yVYYEI	yPEWMS	yEIFTW	yTYDQE	yEWAFa	yDWQEW
yEAWLG	yMDWIT	yDWWAE	yTYDVE	yEWAwV	yGTTYA
yVAWIE	yMDFIS	yIEWFH	yHWDII	yWFAME	yPHHWV
ySMWID	yIEWSR	yAIWFI	yEWETW	yENIwW	yTYGEI
yQVWFA	*yIDWTG	yQVWWI	yVYESY	yNSVFR	yWIGII

<sup>a</sup>M, Nle; Y, F<sub>2</sub>Y; y, pY; \*, sequences selected for kinetic analysis.

Table S6. Potential VH1 substrates from database search

Protein	Phosphorylation Site	Sequence <sup>a</sup>														
		R	G	k	s	F	t	L	t	I	t	V	F	T	N	P
AML2	T153	R	G	k	s	F	t	L	t	I	t	V	F	T	N	P
ASK1	T842	C	T	E	t	F	T	G	t	L	Q	Y	M	A	P	E
BORA	S252	K	L	D	t	F	C	G	s	P	P	Y	A	A	P	E
Casp2	S139	K	I	A	D	F	G	W	s	V	H	A	P	s	s	R
CYP3A4	S116	L	N	T	S	Y	P	L	s	P	L	S	D	F	A	T
ERF	S251	D	P	Y	E	F	L	Q	s	P	E	P	A	A	S	A
<b>ERK5</b>	T219	A	E	H	Q	y	F	M	t	E	y	V	A	T	R	W
<b>JNK1</b>	T183	A	G	t	S	F	M	M	t	P	y	V	V	t	R	Y
JNK2	T183	A	C	T	N	F	M	M	t	P	y	V	V	t	R	Y
JNK3	T221	A	G	t	S	F	M	M	t	P	y	V	V	t	R	Y
MAD1L1	S29	D	S	L	S	Y	y	H	s	P	A	D	S	F	S	S
MARCKS	S118	I	P	N	G	F	G	T	s	P	L	t	P	S	A	R
Myf-5	S133	K	K	L	E	F	S	S	s	P	D	S	L	S	T	I
NCAPG	T308	L	N	A	L	F	S	I	t	P	L	S	E	L	V	G
PASK	T1165	L	F	Y	t	F	C	G	t	I	E	Y	C	A	P	E
SREBP-1	S439	K	L	D	t	F	C	G	s	P	P	Y	A	A	P	E
TARDBP	S404	T	P	D	G	F	s	C	s	s	s	L	s	A	L	s
TDP1	S563	D	G	T	E	F	G	G	s	I	y	Q	k	V	N	K

<sup>a</sup>The pS/pT residue is shown in boldfaced, lower-case letters.