Supplementary Figure 1

A

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-WT
E-WT	L	F	S	I	S	T	R	Q	H	V	TGWETWV
E-WTa	L	I	K	L	V	G	A	K	M	V	
E-WTb	L	I	S	I	V	A	K	R	I	V	
E-WTc	L	I	M	I	V	A	K	N	E	V	
E-WTd	L	I	K	L	V	K	A	R	L	I	
E-WTe	L	I	R	I	T	A	D	K	L	L	
E-WTf	L	I	M	I	R	A	K	K	L	E	
E-WTg	F	I	M	V	V	A	A	D	L	V	
E-WTh	L	F	T	V	V	G	R	R	L	V	
E-WTi	L	V	M	L	T	T	K	K	L	V	
E-WTj	L	F	S	V	V	R	S	K	L	V	
E-WTk	L	F	S	I	V	A	N	R	H	A	
E-WTl	L	I	S	I	T	N	K	D	F	V	
E-WTm	L	I	R	L	I	A	E	A	M	I	
E-WTn	L	L	R	I	V	A	E	K	L	V	
E-WTo	L	V	S	L	I	K	N	N	L	V	
E-WTp	L	I	S	I	V	S	A	R	M	K	
E-WTq	L	I	M	V	T	T	N	K	L	V	
E-WTr	L	I	M	I	T	T	K	R	L	V	

B

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-35
E-35	I	I	S	V	V	E	T	A	Q	E	SKLRFWV
E-35a	F	I	M	V	V	A	A	D	L	V	
E-35b	L	I	M	I	V	N	E	N	L	A	
E-35c	L	I	M	V	T	T	N	K	L	V	
E-35d	I	I	M	I	I	A	E	D	I	V	
E-35e	I	I	M	I	I	A	K	D	I	V	
E-35f	L	I	M	L	I	E	A	K	L	V	
E-35g	L	V	M	I	V	G	D	D	L	A	
E-35h	I	I	M	I	A	T	A	D	M	I	

C

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-39
E-39	F	F	S	I	I	G	T	D	S	E	FFGERWV
E-39a	F	I	T	I	M	G	K	D	L	V	
E-39b	L	F	S	I	V	G	R	H	L	V	
E-39c	L	V	S	L	I	K	N	N	L	V	
E-39d	I	I	S	I	V	K	A	N	L	V	
E-39e	L	I	T	I	I	G	R	R	L	E	
E-39f	L	I	S	I	V	A	T	L	L	A	
E-39g	F	F	S	I	I	A	R	R	V	E	
E-39h	L	I	S	I	I	A	A	R	L	A	
E-39i	L	F	S	I	T	T	R	H	F	V	
E-39j	F	F	S	I	I	G	R	N	F	V	
E-39k	L	I	S	I	V	N	G	N	L	V	
E-39l	L	F	S	I	V	N	R	L	V	Q	
E-39m	L	F	S	I	I	R	R	K	I	Q	

D

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-41
E-41	F	F	R	I	V	A	A	D	Y	T	GNRDGWV
E-41a	L	I	R	L	T	A	D	R	L	I	
E-41b	F	F	R	I	V	A	A	K	Y	L	
E-41c	L	I	R	V	T	T	E	R	L	I	
E-41d	I	I	R	V	T	T	D	S	L	I	
E-41e	L	I	K	L	V	S	E	R	L	I	
E-41f	L	I	R	I	I	A	E	L	M	V	
E-41g	L	I	R	I	V	N	E	N	L	I	
E-41h	I	I	R	L	V	A	A	R	L	I	
E-41i	I	I	R	I	I	E	A	R	L	V	
E-41j	I	I	R	I	M	E	E	D	F	L	
E-41k	F	F	R	I	I	A	K	A	L	V	
E-41l	L	I	R	I	I	A	K	L	M	V	
E-41m	L	I	R	I	T	A	E	R	Y	I	
E-41n	I	I	K	I	V	T	E	R	L	V	

E

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-47
E-47	L	F	K	V	V	R	E	M	G	Q	SLDGWWV
E-47a	I	I	M	I	A	T	A	D	M	I	
E-47b	L	I	K	L	V	A	D	A	L	V	
E-47c	L	I	M	I	A	T	E	K	L	A	
E-47d	L	F	K	I	M	S	A	K	L	A	
E-47e	L	I	M	V	T	T	N	K	L	V	
E-47f	I	I	M	I	A	T	A	N	M	I	
E-47g	F	F	K	V	V	E	E	R	L	V	
E-47h	L	I	K	I	T	T	D	R	L	I	
E-47i	L	I	K	V	I	A	A	D	M	L	
E-47j	I	I	K	I	T	T	A	R	M	L	
E-47k	L	I	R	I	A	T	A	K	I	I	

F

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-12
E-12	L	F	K	I	M	T	R	K	L	E	SNHGRWV
E-12a	F	I	M	V	V	A	A	D	L	V	
E-12b	I	I	M	I	I	A	A	T	L	A	
E-12c	L	I	M	I	M	T	N	S	L	V	
E-12d	F	I	K	I	V	A	E	N	E	V	
E-12e	L	I	R	I	M	A	A	K	L	V	
E-12f	V	I	T	I	T	A	A	H	L	A	
E-12g	L	I	M	I	V	A	K	N	E	V	
E-12h	L	I	K	L	V	G	A	K	M	V	
E-12i	I	I	K	I	V	A	A	N	V	V	
E-12j	I	V	T	I	T	A	R	Q	L	V	
E-12k	I	I	S	I	T	A	G	A	L	A	
E-12l	F	I	T	I	M	A	R	K	L	A	
E-12m	L	I	S	I	V	A	K	R	I	V	
E-12n	I	I	T	I	T	T	N	H	I	V	

G

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	
E-19	F	F	T	V	I	A	D	I	L	V	P-19
E-19a	I	I	T	I	V	G	R	E	L	A	RYGGFRV
E-19b	I	I	I	V	D	T	A	D	L	A	
E-19c	F	I	M	V	V	A	A	D	L	V	
E-19d	I	I	M	I	V	A	K	K	L	A	
E-19e	L	I	M	I	I	K	E	K	L	A	
E-19f	L	L	T	I	I	A	N	D	I	V	
E-19g	I	I	M	I	A	T	A	D	M	I	P-19a
E-19h	I	I	S	I	I	N	D	R	L	A	SLWKFWV
E-19i	F	I	M	V	V	A	A	D	L	V	
E-19j	L	I	M	I	I	A	E	I	L	A	
E-19k	L	I	M	V	T	T	N	K	L	V	
E-19l	L	I	N	I	T	T	E	K	L	V	
E-19m	F	F	M	I	T	A	E	K	L	A	
E-19n	I	I	S	I	V	T	E	K	L	V	
E-19o	F	I	M	I	V	A	A	K	L	A	
E-19p	L	V	S	I	I	A	D	R	L	L	
E-19q	F	F	K	V	V	E	E	R	L	V	
E-19r	I	I	S	I	V	S	D	A	L	A	
E-19s	L	I	M	I	V	A	E	E	L	A	
E-19t	L	V	R	I	T	D	T	N	L	V	
E-19u	L	I	M	I	I	A	K	I	L	A	
E-19v	I	I	M	I	A	T	A	N	M	I	
E-19w	F	I	S	I	V	T	E	R	L	V	
E-19x	L	V	M	I	V	A	K	K	L	A	

H

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-14
E-14	I	F	R	I	A	T	R	S	L	I	RWESDWL
E-14a	L	V	R	I	V	S	A	H	L	K	
E-14b	L	F	K	I	M	S	A	K	L	A	
E-14c	L	I	I	I	E	R	A	K	L	K	
E-14d	L	I	T	I	S	T	R	D	M	V	
E-14e	L	I	R	I	T	T	R	L	L	I	
E-14f	I	I	T	I	A	A	K	K	L	V	
E-14g	I	I	T	I	V	G	K	K	L	K	
E-14h	F	F	R	I	M	S	A	R	L	I	
E-14i	L	I	R	I	T	N	E	N	L	K	
E-14j	L	I	R	V	V	S	E	R	L	K	
E-14k	I	I	K	I	T	A	K	R	L	K	
E-14l	I	I	R	I	A	T	A	N	I	V	
E-14m	I	I	K	I	A	T	E	H	F	V	
E-14n	I	I	T	I	V	K	R	R	L	I	
E-14o	I	I	T	I	S	S	R	N	L	V	

I

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-48
E-48	L	F	N	L	V	A	D	K	Q	E	WVFGPWL
E-48a	F	I	R	I	M	A	E	N	L	I	
E-48b	L	L	K	I	T	A	A	H	L	I	
E-48c	L	I	K	L	M	A	A	K	L	V	
E-48d	L	I	R	L	V	A	G	K	L	V	
E-48e	I	I	K	I	A	T	D	R	L	V	
E-48f	L	I	K	L	V	A	K	K	L	V	
E-48g	L	I	M	V	T	T	N	K	L	V	
E-48h	L	I	M	I	V	N	A	K	L	A	
E-48i	L	I	K	I	M	A	A	R	L	E	
E-48j	L	V	M	L	V	A	K	R	L	V	
E-48k	L	I	R	L	I	A	S	R	L	E	
E-48l	L	I	M	L	V	A	A	R	L	A	
E-48m	L	I	M	I	A	A	A	H	L	I	

J

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	
E-55	F	F	T	I	V	S	G	K	E	I	P-37
E-55a	L	I	K	L	V	G	A	K	M	V	HYRFWDV
E-55b	L	I	T	L	T	A	G	A	I	V	
E-55c	L	I	S	I	V	A	K	R	I	V	
E-55d	F	I	K	I	V	A	E	N	E	V	
E-55e	V	I	T	I	T	A	A	H	L	A	
E-55f	L	I	M	I	V	A	K	N	E	V	

K

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-37a
E-37	L	I	T	I	V	S	S	R	E	Q	LYRHWRV
E-37a	I	I	M	I	A	T	A	D	M	I	
E-37b	L	I	T	I	V	A	A	D	D	A	P-37
E-37c	L	F	R	I	T	T	R	R	L	A	PYRYFDV
E-37d	L	I	T	I	M	A	A	R	L	A	
E-37e	L	I	M	I	T	A	K	R	L	A	
E-37f	L	I	T	I	A	T	R	K	I	V	
E-37g	L	I	R	I	V	K	T	N	L	A	
E-37h	L	I	R	I	V	T	G	K	L	V	
E-37i	F	I	T	I	T	T	A	V	L	A	
E-37j	L	I	R	I	T	T	G	Q	L	A	
E-37k	I	V	R	I	T	T	K	D	L	A	
E-37l	L	I	R	I	E	A	G	N	L	A	

L

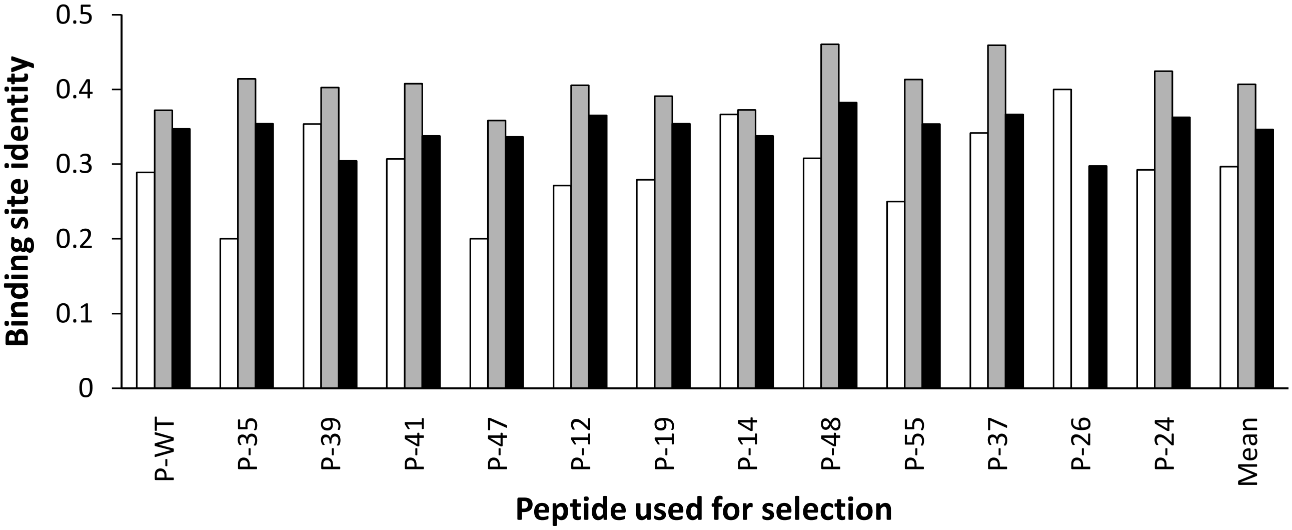
PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-26
E-26	V	I	T	I	V	D	N	D	L	E	FWDGRRV
E-26a	I	I	T	I	I	A	K	R	I	E	

M

PDZ	PDZ binding site										Peptide
	23	25	26	27	28	48	49	51	79	83	P-24
E-24	F	F	I	L	G	T	A	R	L	V	WFTSGWM
E-24a	L	I	M	I	V	T	A	R	L	I	
E-24b	L	I	M	I	V	T	A	K	Y	V	
E-24c	L	I	K	L	M	A	A	K	L	V	
E-24d	L	I	M	V	T	T	N	K	L	V	
E-24e	L	I	K	I	M	A	A	R	L	E	
E-24f	L	V	M	L	V	A	K	R	L	V	
E-24g	L	I	R	V	M	G	A	D	L	V	
E-24h	F	I	R	I	M	A	E	N	L	I	
E-24i	F	F	K	V	M	A	A	K	L	I	
E-24j	L	I	K	I	M	S	S	L	L	I	
E-24k	L	L	K	I	T	A	A	H	L	I	
E-24l	F	I	K	I	M	S	A	R	L	E	
E-24m	L	I	R	I	V	S	A	K	M	I	

Supplemental Fig. S1. Synthetic PDZ domains selected for binding to peptide ligands. The sequence is shown for the 10 positions that were varied in the Erbin-PDZ library and residues that were conserved as the wt are shaded in grey. The peptide against which each set of domains was selected is shown to the right. Only unique domain sequences are shown.

Supplementary Figure 2



Supplemental Fig. S2. Comparison of binding sites of synthetic PDZ domains selected for binding to various peptides. The binding site identity (*y* axis) was calculated by comparing sequences at the ten positions that were varied in the Erbin-PDZ library. For domains selected for binding to each peptide (*x* axis), the following mean binding site identities were calculated: the reference domain compared with all unique evolved domains selected against the corresponding optimal peptide ligand (white bars), all unique evolved domains selected against the same peptide compared to each other (grey bars), all unique evolved domains selected against the same peptide compared to all unique evolved domains selected against other peptides (black bars). For peptide P-26, only one evolved domain was obtained, and thus, the grey bar is absent.