Supplementary Table 3. Classification of arrayed peptides according to their relative binding to the wt and Y65C mutant PQBP1 WW domain.

Class 1	Y65C mutant reactivity is less than wt				
SPOT#	sequence	wt reactivity	mut reactivity	log ratio	ranking score
3	RPPRLLPPRPGP	1.6948	1.2824	-0.4124	836
7	PPPPPPLPFACC	2.2982	1.7816	-0.5166	651
8	PPPPPPLPFQTS	1.5604	0.6128	-0.9476	184
23	DSLCLPRQRPPM	1.5289	1.0970	-0.4320	791
34	LPSLPPLPARCL	2.4433	1.6850	-0.7583	337
54	AAPCPPRPWQLG	2.1023	1.5355	-0.5669	580
57	LPAAPSPSRPWF	1.5571	1.2045	-0.3527	945
71	LRFGPPAYRRFS	1.4538	0.8036	-0.6502	480
86	NSRCPPAYTMVG	1.3693	0.8914	-0.4779	710
87	PVYSPPAYHMHV	1.1952	0.9273	-0.2678	1102
90	RPVFPPAYWTSP	1.5458	1.2351	-0.3107	1024
93	YRAAPPAYGRGG	2.1335	1.8655	-0.2681	1100
124	VPKSPPAYARSS	1.4307	0.7086	-0.7221	379
144	SCQLPPAYYDTR	1.4232	1.1584	-0.2648	1109
149	APSRPPAYQAMG	2.0258	1.5763	-0.4494	752
159	GPGIPPAYSSPR	1.9002	0.6531	-1.2472	43
160	LRHSPPAYSGPA	1.8791	0.8153	-1.0638	119
161	RFKEPPAYGPMC	2.3271	1.8771	-0.4500	750
162	PAPRPPAYNRAL	2.2517	1.3390	-0.9127	208
164	SGPLPPAYSPRY	1.9631	0.5637	-1.3994	18
176	PPGPPPAYAPTN	1.7490	0.9487	-0.8003	296
177	SGPPPPAYHYRN	1.7619	1.2744	-0.4875	695
178	PPPPPPAYNYGS	1.9788	1.0810	-0.8978	216
180	EPPPPPAYRTGC	1.6912	0.9280	-0.7632	329
181	PPPGPPAYDPYG	2.3449	1.4718	-0.8731	236
190	LKRVPPCYLGNS	2.1821	1.7554	-0.4267	805
196	PGQAPPCYAPTY	1.5852	1.0476	-0.5377	621
197	PCNRPPCYVPCT	2.5307	2.1514	-0.3793	889
213	HRYRPPDYRDLI	2.1105	1.6815	-0.4291	801
233	CARGPPDYGDYR	2.7398	2.4543	-0.2856	1068
251	PPRPPPDYLRGG	2.2011	1.6635	-0.5376	622
279	RISCPPDYWGQG	1.0810	0.6659	-0.4151	828
305	APPPPPDYHHHH	2.1832	0.8842	-1.2990	32
316	VASRPPDYYYGM	2.0502	0.9027	-1.1475	78
327	LCIGPPEYQEKK	1.3629	1.0864	-0.2765	1087
386	PRGPPPEYPFKT	2.7726	1.5360	-1.2366	52
387	HRGPPPEYPFKG	2.2656	1.3467	-0.9188	207
403	SPTPPPFYYGLG	1.2447	0.2572	-0.9874	162
414	KHPGPPFYVPHA	0.9655	0.6064	-0.3591	928
425	LPARPPFYAPAH	2.8572	2.1057	-0.7515	343
427	SPPFPPFYSTKP	1.0232	0.7338	-0.2894	1058
428	PPPFPPFYSTKP	1.7080	1.3649	-0.3432	961
429	SPPFPPFYSTKL	1.1819	0.5596	-0.6223	506
454	GGLKPPGYYVQR	1.5112	1.2000	-0.3112	1022
508	CARAPPGYCSST	2.4813	2.1671	-0.3142	1017
529	VAGSPPGYWGQG	1.2067	-0.0391	-1.2458	44
539	AMGAPPGYRPSA	1.3798	0.5909	-0.7889	303
545	HAPYPPGYQRHF	1.5565	1.1323	-0.4242	812
549	YPSLPPGYQNIT	1.6904	0.7010	-0.9894	161

550	YPSLPPGYQNTT	2.0284	1.1210	-0.9074	211
553	KKKRPPGYYSYL	1.3611	1.0021	-0.3590	929
566	GLRGPPGYTFGS	1.4199	0.5296	-0.8902	227
569	FSNRPPGYPSQP	2.4151	1.3152	-1.0999	96
570	IQNRPPGYPNMN	2.0556	0.8588	-1.1968	64
575	LGPPPPGYHPFL	2.1694	1.6386	-0.5308	635
585	QRLPPPGYGRPP	1.2908	0.7872	-0.5036	682
587	RTGTPPGYRGTS	1.9654	1.1885	-0.7769	313
589	AHGPPPGYGCAA	2.3328	1.3638	-0.9690	174
590	SFGTPPGYGCAA	1.5207	0.5930	-0.9277	201
601	PCQPPPGYAPVP	1.4120	0.6173	-0.7947	300
603	RSSPPPGYVPER	1.6829	0.9245	-0.7583	336
605	PSVPPPGYMPRQ	1.1814	0.1528	-1.0286	137
610	LPSCPPGYFDAR	2.4530	2.1766	-0.2765	1086
612	PPMPPPGYFGQA	1.5769	1.0724	-0.5045	679
616	FPPPPPGYNASS	1.1930	0.4885	-0.7046	401
617	LPPPPPGYPAPK	2.0793	1.1042	-0.9751	170
623	GSAGPPGYPGPR	2.1665	0.9902	-1.1762	71
624	GPQGPPGYGKMG	2.7200	1.6104	-1.1096	92
625	GPPGPPGYGPAG	2.3301	1.0409	-1.2892	35
626	GPPGPPGYGSQG	2.7054	1.3045	-1.4009	17
627	GPAGPPGYGPQG	2.1064	1.2376	-0.8688	240
628	GPPGPPGYKGFP	3.0029	2.2602	-0.7427	355
629	GPPGPPGYPGKQ	2.8925	1.9069	-0.9856	164
630	GPPGPPGYCDSS	2.1651	1.0971	-1.0680	116
631	GARGPPGYRGPT	2.0400	1.2574	-0.7826	306
635	MYPCPPGYPYPP	1.3935	0.8211	-0.5724	568
649	TCTCPPGYGGFH	1.2458	0.7894	-0.4564	741
653	RCICPPGYAGDH	1.5099	1.2193	-0.2906	1055
659	LCSCPPGYQGRS	2.4013	1.9509	-0.4504	749
662	HCHCPPGYTGRY	2.7078	2.4197	-0.2881	1064
664	LCKCPPGYSGTI	2.0641	1.6038	-0.4604	735
666	CGACPPGYSGNG	1.2898	0.6126	-0.6772	433
673	IGLSPPHYGHYG	2.1369	1.7401	-0.3968	861
678	FQALPPHYGRPY	1.1605	0.7945	-0.3659	916
683	YGPPPPHYEGRR	2.6210	2.2341	-0.3869	880
684	KPPLPPHYTSTA	1.4168	0.5559	-0.8608	246
689	PPSSPPHYQRVP	1.7328	1.1400	-0.5929	544
691	RTPGPPHYPPPH	2.7164	2.1046	-0.6118	518
694	PSRPPPHYPGIK	2.6821	1.7787	-0.9035	213
695	PSRPPPHYPGIK	2.7130	1.9611	-0.7519	342
696	PPPPPPHYPVLQ	2.0269	0.9989	-1.0280	138
697	PWPPPPHYSIAL	1.5382	0.8046	-0.7337	370
715	GGRAPPHYGYTF	1.7119	1.3733	-0.3386	968
753	PSIHPPIYPSIH	1.3944	0.7759	-0.6185	511
754	PSIHPPIYPCIH	1.4415	1.0116	-0.4299	797
774	KRGRPPKYNAVL	2.0582	1.7257	-0.3324	979
778	LDGCPPKYAKTL	1.6322	1.3625	-0.2697	1098
805	YRTNPPKYPSKQ	1.8873	1.5994	-0.2879	1066
810	YGSSPPKYTFGA	2.3278	1.8923	-0.4355	783
813	EVLCPPKYLKLS	1.3328	1.0771	-0.2557	1124
828	LPPFPPKYYLAM	1.1263	0.7328	-0.3935	873
863	AFRHPPLYPGPA	1.5502	0.9513	-0.5989	535
915	CPRPPPLYLMVP	2.2203	1.5594	-0.6609	462

917	VPRPPPLYSHRM	2.7673	2.2816	-0.4858	698
928	RHSNPPLYPSRP	1.4409	0.9804	-0.4605	733
929	VHPPPPLYPLRP	1.1825	0.4076	-0.7749	315
930	PVPPPPLYPPPP	1.2977	0.0143	-1.2834	39
931	PPPPPPLYPRRG	2.3037	1.5043	-0.7994	297
932	PPPPPPLYQRVS	1.8357	1.2014	-0.6343	495
936	LLPPPPLYTRHR	1.8882	1.3379	-0.5504	602
938	QLPPPPLYRPRW	1.6464	0.5811	-1.0653	118
946	RSSFPPLYSSGL	1.1166	0.4482	-0.6685	449
950	WSPSPPLYEEYR	1.3648	0.6389	-0.7259	377
951	SPKYPPLYPPGR	2.0156	1.2410	-0.7745	316
984	YGSSPPMYTFGQ	1.2034	0.5084	-0.6950	408
987	CARRPPMYHFDY	2.5240	2.2050	-0.3190	1007
996	GSPGPPNYFDYW	1.0840	0.4319	-0.6521	473
1006	PGPAPPNYSRPH	1.0771	0.8079	-0.2692	1099
1021	PTFIPPNYRVKV	1.8543	1.5091	-0.3452	958
1037	RVIRPPNYAGPL	0.8995	0.5542	-0.3453	957
1053	FRKAPPNYAMKK	2.2814	1.5283	-0.7531	340
1076	PYGRPPPYDRGD	1.6638	1.3389	-0.3249	995
1077	RHYMPPPYASLT	1.8264	1.5085	-0.3179	1009
1082	PTFIPPPYGVLR	1.2434	0.9816	-0.2618	1114
1086	VSFPPPPYLARS	1.0523	0.2911	-0.7612	333
1096	GPCLPPPYVRTA	2.2980	1.7645	-0.5335	631
1114	TRNSPPPYGFHL	1.3864	0.8799	-0.5065	671
1118	ARARPPPYTGVW	1.7777	1.5217	-0.2559	1122
1119	GRKYPPPYKHFW	1.7687	1.2841	-0.4846	699
1131	QHHTPPPYYSRP	2.2660	1.1809	-1.0851	106
1132	HHGSPPPYNQRP	2.6235	2.2347	-0.3888	878
1136	TVLPPPPYRHRN	1.5216	1.1821	-0.3395	967
1141	EGLWPPPYRPRR	1.3939	0.9507	-0.4432	770
1144	FHDPPPPYTAYK	1.2656	0.4256	-0.8400	265
1153	AVWQPPPYGATK	1.2188	0.6812	-0.5376	623
1157	GAGSPPPYRRRK	1.6192	0.8385	-0.7807	309
1162	KRPPPPPYNCRY	2.9644	1.9819	-0.9824	165
1166	GPGSPPPYKMIQ	1.9299	0.5778	-1.3522	24
1175	QRPQPPPYWRGP	2.0956	1.0167	-1.0789	111
1177	GYPTPPPYPFTP	2.3154	1.5351	-0.7803	310
1181	FPRAPPPYPTHH	2.8872	2.1094	-0.7778	312
1183	TRHPPPPYNTAK	1.8696	1.0250	-0.8446	261
1193	APLSPPPYLPHR	1.7814	0.4800	-1.3014	31
1198	WVPGPPPYLPRQ	2.3894	1.5811	-0.8083	290
1208	VVAPPPPYMHPW	2.0160	0.6131	-1.4029	16
1214	RYSPPPPYSSHS	2.8779	2.2060	-0.6719	442
1220	RALPPPPYTTFP	1.9233	1.2214	-0.7018	403
1221	SPPGPPPYGKRA *	3.1702	2.4038	-0.7664	327
1222	APSPPPPYPVTP	2.3424	0.6328	-1.7097	1
1228	SPPAPPPYKAPR	1.7057	0.6160	-1.0897	105
1229	PGRIPPPYSCTP	1.6252	0.9892	-0.6360	493
1233	LCIQPPPYPGNI	1.9551	1.1865	-0.7686	321
1234	YPAGPPPYGHDH	2.3976	1.5592	-0.8384	267
1235	SYPGPPPYGHDH	1.8848	0.5235	-1.3613	21
1237	VRGPPPPYQMTP	2.5763	1.3366	-1.2397	49
1238	VRGPPPPYHSKP	2.7399	1.5516	-1.1883	67
1239	PHQQPPPYPLMH	1.6659	0.7935	-0.8724	238

1242	VCTPPPPYPPSW	2.2796	1.5366	-0.7430	354
1246	HCTPPPPYHADP	1.4237	0.3054	-1.1182	90
1249	YQGPPPPYPKHL	2.5466	1.8711	-0.6755	437
1250	VPSPPPPYTTSH	2.6962	1.3695	-1.3268	27
1259	LGHWPPPYPVPR	1.4672	0.8760	-0.5912	547
1260	PYRSPPPYVPPA	1.5960	0.5479	-1.0481	128
1262	FTRPPPPYPGNI	1.9072	0.2529	-1.6543	3
1264	QRSPPPPYPGNA	1.7287	0.5196	-1.2091	62
1267	GPPRPPPYGYQR	2.6664	2.2250	-0.4414	771
1268	QKSPPPPYSRLS	1.7532	0.6560	-1.0972	100
1273	CPLPPPPYQNQS	2.4478	1.7356	-0.7123	389
1274	CPPPPPPYQNQS	3.0006	2.1638	-0.8368	269
1275	APPPPPPYRMHG	2.9955	2.2341	-0.7614	331
1276	PPGPPPPYHAHA	3.1389	2.1434	-0.9955	154
1277	PQGPPPPYHAHA	2.3900	1.1275	-1.2625	41
1282	PPSPPPPYDSRL	2.5199	1.0495	-1.4704	12
1283	PPTPPPPYSAFQ	2.3822	1.4618	-0.9205	206
1284	PHPQPPPYGHCV	3.0622	2.3888	-0.6734	441
1285	SGPQPPPYGHCV	2.5206	1.6299	-0.8908	226
1286	PHPHPPPYPHFT	2.9491	2.4564	-0.4927	690
1287	AAPPPPPYPYLV	2.3514	1.0720	-1.2794	40
1288	LGPYPPPYPPPA	2.7600	1.4047	-1.3553	23
1289	PSHPPPPYPFGS	2.5714	1.8357	-0.7357	367
1290	PPYPPPPYPSGE	1.6214	0.5473	-1.0741	113
1292	PPPPPPPYSGCA	2.3888	1.1520	-1.2368	51
1296	PPPPPPPYGRLL	2.1650	0.9547	-1.2104	61
1297	GPPPPPPYGRLL	2.2325	0.8755	-1.3570	22
1298	PVPPPPPYLASY	2.2594	0.6566	-1.6028	5
1299	PPPPPPPYYPSF	2.5154	1.2738	-1.2416	48
1300	PPPPPPPYGPGR	2.7552	1.6637	-1.0915	104
1301	PPSPPPPYGPGR	3.0040	2.2105	-0.7934	301
1302	PPPHPPPYGPGR	2.9184	1.9791	-0.9393	194
1303	PPPPPPPYLASL	2.2778	0.9577	-1.3201	29
1304	KLPPPPPYGRLL	1.8838	1.3104	-0.5734	565
1306	PQPPPPPYGHKR	2.4607	1.4918	-0.9690	175
1307	FPPPPPPYFPES	1.7698	0.5547	-1.2151	58
1308	LPPPPPPYNHPH	2.4242	1.2898	-1.1344	83
1309	LPPPPPPYHPPA	2.1123	1.0300	-1.0823	109
1310	LFRPPPPYPRPR	2.4402	2.0654	-0.3748	899
1311	VYRPPPPYPPPR	2.7876	2.2820	-0.5057	676
1314	RISPPPPYPPPK	2.4505	1.5073	-0.9432	189
1317	LAPPPPPYRQPP	2.5070	0.8234	-1.6836	2
1318	PPSLPPPYPQRH	2.6430	1.6069	-1.0361	133
1319	PPYFPPPYPQPP	2.2840	1.2822	-1.0018	152
1320	PPYFPPPYQQLA	1.7586	1.0817	-0.6769	434
1321	PPYFPPPYQPIY	1.6937	0.9761	-0.7176	384
1322	PPYFPPPYQPLP	1.8071	0.7845	-1.0225	143
1323	PGPAPPPYLPPQ	1.7386	0.6780	-1.0606	121
1337	CASAPPQYSLLK	2.4973	1.7286	-0.7687	320
1341	YPCCPPQYCLNQ	1.3823	0.9224	-0.4599	736
1354	CELWPPQYPSKG	1.9242	1.3769	-0.5473	606
1358	FSCKPPQYGQLE	1.9457	1.5843	-0.3614	924
1359	RSCTPPQYGGRP	2.6709	2.3445	-0.3263	989
1372	KPGSPPQYLLRY	1.9281	1.1759	-0.7522	341

1373	KPGSPPQYLLKY	1.5662	1.2769	-0.2893	1059
1378	KPVSPPQYLLRY	1.2354	0.8696	-0.3658	918
1379	SRGPPPQYPHVV	1.5588	1.1299	-0.4290	802
1384	RGRNPPQYQRSP	1.2840	0.8366	-0.4474	758
1390	PRLSPPQYSHQV	1.8566	0.9130	-0.9436	188
1416	PLRKPPRYSDLH	1.5934	1.2382	-0.3552	935
1421	TQLRPPRYIHKA	1.9296	1.3594	-0.5702	573
1425	GFPGPPRYRSAA	2.9447	2.5014	-0.4432	769
1430	PFPQPPRYPLML	1.4452	0.9895	-0.4557	743
1449	RFSHPPRYWPVL	1.3987	1.0606	-0.3381	970
1451	RASSPPRYLQHD	1.8863	1.2436	-0.6426	486
1458	ATGCPPRYECSA	1.5880	1.0922	-0.4958	687
1459	CARDPPRYCSAG	2.6930	2.2613	-0.4317	793
1462	VTVAPPRYLPHS	1.5466	1.1408	-0.4058	846
1466	PGSPPPRYQGPV	1.1236	0.2743	-0.8494	256
1470	RPFFPPRYAAAA	1.3056	1.0259	-0.2797	1085
1472	YGSSPPRYSFGA	2.1327	1.6671	-0.4656	722
1482	SSSAPPRYSPPY	1.8471	1.0792	-0.7679	324
1483	PPPPPPRYDPCA	2.6760	1.8391	-0.8369	268
1485	PPSRPPRYSSSS	3.0611	2.4362	-0.6249	504
1489	RAPLPPRYSNQN	2.0515	1.6043	-0.4472	760
1493	RPPMPPRYPAPY	2.7842	2.3365	-0.4477	757
1494	VPPLPPRYRFRD	2.5407	2.1509	-0.3897	876
1522	SYCEPPSYRPMH	2.7479	2.4215	-0.3264	988
1542	CDLLPPSYSKTQ	1.3274	0.9134	-0.4140	832
1547	DCRPPPSYPGSG	0.9220	-0.1370	-1.0590	123
1552	GFPKPPSYNVAT	1.7702	0.8742	-0.8960	219
1572	TRGPPPSYGGSS	1.2507	0.5831	-0.6676	452
1587	GGPHPPSYSLPP	1.3212	0.2136	-1.1076	93
1601	KVPYPPSYVPAV	1.0552	0.4534	-0.6018	528
1602	AQHRPPSYHPSQ	1.9125	1.5139	-0.3987	854
1606	ASCSPPSYAGLG	0.9401	0.6320	-0.3081	1029
1627	QKPAPPSYQRWL	1.1769	0.8793	-0.2976	1047
1628	PKPAPPSYQRWR	2.3665	1.5470	-0.8194	283
1629	QKPAPPSYQRWR	1.9076	1.5841	-0.3236	998
1630	PGPRPPSYSRAA	1.8578	1.2061	-0.6517	476
1631	RRPGPPSYEAHL	1.6044	1.1661	-0.4383	775
1643	YKTPPPSYNSAL	1.1425	0.0711	-1.0714	115
1677	PSAPPPSYAKVI	1.2856	0.1014	-1.1842	69
1686	VYVPPPSYRSPP	0.8088	0.3616	-0.4472	761
1693	PPAPPPSYGATH	1.1044	0.3350	-0.7694	319
1696	LGPPPPSYTCFR	2.0149	1.5928	-0.4221	816
1697	LPPPPPSYPSPK	1.6096	0.6501	-0.9594	177
1698	AYPPPPSYPSPK	1.7295	0.7488	-0.9808	166
1699	LAPPPPSYPCGP	1.5654	0.5495	-1.0160	145
1702	PPSPPPSYHPPP	1.6120	0.3668	-1.2452	46
1703	PPQPPPSYYPPT	1.5343	0.6476	-0.8867	229
1704	PPPPPPSYSTLA	1.1741	0.3290	-0.8451	260
1705	PPPPPPSYMHIP	1.9099	0.4352	-1.4748	10
1707	QPPPPPSYSPAR	1.4525	1.0554	-0.3972	859
1729	SPRLPPTYMHAM	1.5784	1.0673	-0.5111	662
1730	ALNRPPTYPTKY	1.3143	0.8533	-0.4609	732
1741	FHKQPPTYVRAQ	1.3004	0.8953	-0.4051	847
1750	PRRGPPTYRADD	0.8593	0.0328	-0.8265	275

1751	RVKCPPTYPDVV	1.4920	0.7470	-0.7450	352
1761	QSYCPPTYSTTG	1.0009	0.4692	-0.5317	632
1765	CARDPPTYSPRA	2.0585	1.8039	-0.2547	1126
1777	CGLIPPTYRGVV	3.0951	2.5322	-0.5629	584
1779	CGLIPPTYRGVR	3.0806	2.7674	-0.3132	1018
1786	RASPPPTYSPSM	1.3572	0.4166	-0.9406	192
1792	PPPPPPTYRTVV	1.9264	0.9318	-0.9946	155
1794	YPPPPPTYNPNF	2.1783	1.3485	-0.8298	274
1798	FPTAPPTYPPYP	1.6524	0.0344	-1.6180	4
1823	HQQRPPVYRRPG	1.9312	1.2344	-0.6968	407
1834	QRSRPPVYECVP	1.9038	1.1356	-0.7682	323
1865	YPAGPPVYNPAA	1.5076	0.0318	-1.4758	9
1870	PPPPPPVYCVCR	1.5492	1.1054	-0.4438	767
1874	VRGPPPVYTNGK	2.5562	1.3078	-1.2484	42
1878	DPARPPWYACSS	1.8501	1.3982	-0.4519	747
1879	TAGRPPWYNEHG	2.1498	1.2636	-0.8862	230
1905	AQGRPPYYTPNQ	2.2705	1.3086	-0.9619	176
1907	HHTPPPYYSRPS	2.4478	1.1242	-1.3236	28
1920	PPPPPPYYPSFL	2.1485	0.9618	-1.1866	68
1929	TPAAPPYYCDIK	1.4252	1.0103	-0.4149	829
1952	ARGCPPYYGMDV	2.2268	1.5091	-0.7178	383
Class 2	**Y65C mutant reactivity is equal to wt**				
SPOT#	**sequence**	**wt reactivity**	**mut reactivity**	**log ratio**	**ranking score**
13	SCTRLPRAQPPL	2.3552	2.2869	-0.0683	1478
14	HRSRLPRAKPPL	1.5312	1.4537	-0.0775	1467
16	CAPGRPRAQPPA	3.1685	2.9679	-0.2007	1249
37	CGLGLPRPPMLL	2.4826	2.3090	-0.1736	1306
48	RLRALPRALFRN	1.1000	1.3310	0.2310	1848
49	PRLRVLPRATGL	1.3382	1.1872	-0.1511	1337
55	CAPCPPRPASPV	1.6802	1.4645	-0.2156	1220
56	ASPCPPRPRRGC	2.5419	2.5965	0.0546	1651
59	AAPCQPRPTHFV	1.0248	1.1352	0.1104	1730
89	RISRPPAYLPRS	2.2405	1.9983	-0.2422	1159
96	GLMRPPAYCDAK	1.2830	1.1555	-0.1275	1381
145	GCLIPPAYEPWR	1.2485	1.3265	0.0780	1688
186	CCPRPPAYPSAG	2.3316	2.3564	0.0247	1622
187	ACPPPPAYCNTP	1.3848	1.2035	-0.1813	1292
191	LRQPPPCYLTAW	0.9922	0.9292	-0.0629	1489
216	WIRNPPDYHQFK	1.0601	1.0786	0.0185	1612
243	VFRCPPDYPIHP	1.5969	1.4400	-0.1568	1328
273	RPACPPDYTLAL	1.7010	1.6884	-0.0126	1563
274	RTACPPDYTLAM	0.6977	0.8237	0.1260	1744
304	FSQCPPDYHHIH	0.9952	0.8182	-0.1770	1299
308	KKFVPPDYKIHR	1.1233	1.2373	0.1140	1734
326	YCEGPPEYKKRK	2.6229	2.8071	0.1841	1806
329	FCAMPPEYKGHQ	1.8998	1.6685	-0.2313	1188
343	KGCKPPEYRNYQ	2.3776	2.3218	-0.0558	1500
384	RYQHPPEYGAAR	0.7653	0.5728	-0.1925	1267
410	YRKGPPFYHASY	2.1434	2.0778	-0.0655	1485
421	HCDNPPFYRFTA	1.8476	1.7522	-0.0955	1432
479	CAVPPPGYALNF	1.2336	1.0182	-0.2154	1222
506	CAKRPPGYSSSW	2.8148	2.7756	-0.0392	1523
507	CAREPPGYCSSA	1.5224	1.5084	-0.0140	1560
542	IHGGPPGYAPYC	1.9800	1.9238	-0.0562	1499

561	LCFHPPGYSEQK	1.8239	1.5926	-0.2313	1187
576	KFLPPPGYAPCY	1.7846	1.5623	-0.2223	1205
577	KFLPPPGYAPCH	2.0175	1.8020	-0.2155	1221
592	SARPPPGYGCAA	2.0003	1.7894	-0.2109	1229
611	LHDCPPGYFGIR	1.5370	1.4455	-0.0915	1445
632	GTRGPPGYRGPT	1.3163	1.2258	-0.0905	1447
637	LCPSPPGYLCTL	1.3572	1.3751	0.0179	1611
638	FCPAPPGYLRQG	2.2339	2.1411	-0.0928	1442
644	ACQCPPGYQKRG	1.7292	1.9556	0.2264	1841
645	RCICPPGYEVKS	1.8656	1.6294	-0.2362	1170
646	RCICPPGYSLQN	1.5423	1.5313	-0.0110	1573
652	RCTCPPGYTGLR	2.2607	2.4899	0.2292	1845
655	HCLCPPGYYGLH	0.9422	0.8233	-0.1189	1395
657	QCSCPPGYRGPS	2.1755	2.2355	0.0600	1664
663	SCTCPPGYTGRN	2.0518	1.8163	-0.2355	1175
672	LCNTPPHYHGFP	2.4060	2.6441	0.2382	1859
674	VTFRPPHYGAYI	0.9981	1.1819	0.1839	1804
702	KPGSPPHYLLRY	1.5355	1.5099	-0.0256	1541
703	CGLAPPHYPTLL	1.9845	1.9081	-0.0764	1468
705	QKFCPPHYPDSQ	1.0375	0.9019	-0.1356	1366
706	RKCVPPHYKEAE	1.6862	1.5557	-0.1305	1377
707	MSFHPPHYLAAR	1.7323	1.7513	0.0190	1613
709	AAAVPPHYKKLF	0.9405	1.0631	0.1226	1739
717	AARAPPHYEQYF	1.0843	0.9258	-0.1585	1322
718	VQCFPPHYEIFK	1.5031	1.2706	-0.2325	1184
725	VKGNPPIYRFWK	0.8197	0.6785	-0.1412	1355
728	CARAPPIYDYVG	1.7128	1.5378	-0.1750	1303
734	KRFTPPIYQPKK	1.3993	1.3444	-0.0550	1502
744	IYKKPPIYKQRE	1.6081	1.7539	0.1458	1765
745	IYRKPPIYKQHA	2.0485	2.2561	0.2076	1826
746	IYRKPPIYRQHA	2.3455	2.2426	-0.1029	1421
749	IYRKPPIYKRHG	2.0708	2.2987	0.2280	1844
755	PSMHPPIYPFIY	0.6506	0.7079	0.0573	1657
766	PGNWPPIYCKSD	0.6152	0.5550	-0.0602	1493
770	SPIRPPKYYTYK	1.9466	2.1229	0.1763	1795
773	RCGRPPKYNASC	2.6526	2.6434	-0.0092	1576
775	RIGRPPKYRKIP	2.0652	1.9658	-0.0994	1427
783	KMRSPPKYRPFL	2.0365	2.2690	0.2325	1850
792	FTRKPPKYERFI	1.9610	1.9061	-0.0549	1503
793	FIRKPPKYERLI	1.1200	1.0097	-0.1103	1411
807	KKKLPPKYPHCQ	1.7340	1.6493	-0.0847	1456
808	CDSRPPKYPRKS	2.8946	2.7689	-0.1257	1385
809	RYNWPPKYTFGQ	1.5652	1.3343	-0.2309	1190
811	YGSSPPKYTFGQ	2.0896	2.1595	0.0699	1676
814	LKMPPPKYVLWS	0.7432	0.8940	0.1508	1771
816	GCRGPPKYGSPG	2.5958	2.5507	-0.0451	1516
830	DGGHPPKYFHNH	0.9921	0.9606	-0.0314	1536
842	KCHTPPLYRMRI	2.6416	2.4010	-0.2407	1162
844	IQFKPPLYRQRY	1.8453	1.7090	-0.1363	1364
893	KGLWPPLYRPRR	1.1311	1.1893	0.0582	1660
909	GKKPPPLYLKFH	0.9240	1.1420	0.2180	1832
956	GYGMPPLYPQTG	0.7703	0.5252	-0.2451	1150
968	LVACPPMYHLMK	1.4052	1.4396	0.0344	1637
993	AQRRPPNYHFRQ	1.9057	2.1325	0.2268	1842

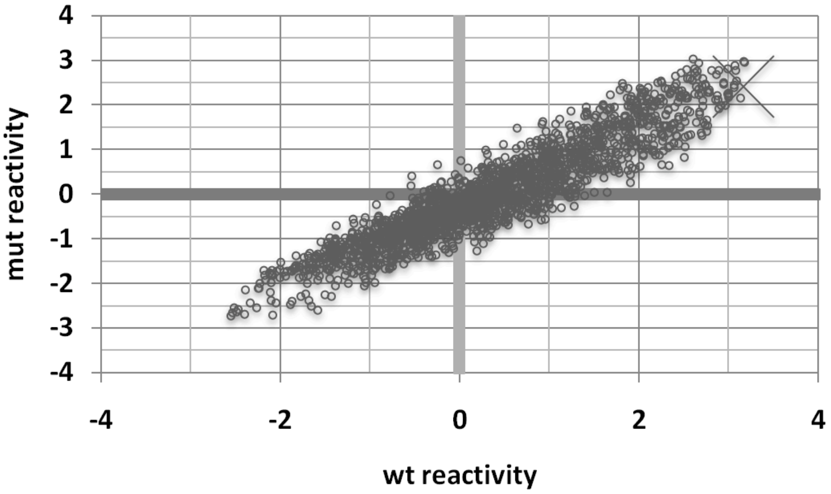
998	SKRPPPNYLCHL	1.5200	1.5003	-0.0197	1550
1011	CKERPPNYPGSL	1.9397	1.8925	-0.0472	1515
1012	CARYPPNYYDSS	2.3993	2.4120	0.0128	1604
1013	CAKFPPNYSNSW	2.7176	2.5865	-0.1311	1376
1016	YGMVPPNYCSQR	1.9795	2.0414	0.0619	1667
1019	RFECPPNYVQVS	1.0007	0.9613	-0.0394	1522
1027	PMFGPPNYAQKF	1.2515	1.1960	-0.0555	1501
1038	RVFRPPNYSGTI	1.2430	1.4868	0.2437	1865
1047	HRKHPPNYKKPV	1.8445	1.7053	-0.1392	1359
1101	LYGCPPPYHTFE	1.0423	1.2447	0.2025	1823
1103	LHGCPPPYHTFE	1.9863	1.7904	-0.1958	1258
1104	LHGCPPPYHTFK	2.9927	2.8066	-0.1862	1279
1116	NSRKPPPYKHIK	1.0279	1.2529	0.2250	1837
1150	PRCSPPPYHPVQ	2.4212	2.4784	0.0572	1656
1225	HDPYPPPYGPHY	1.2290	0.9964	-0.2326	1183
1248	RRCPPPPYPKHL	2.8688	2.6760	-0.1928	1264
1330	GARSPPQYCGTK	1.8834	1.9922	0.1087	1726
1335	GCSFPPQYSALQ	2.0259	2.2098	0.1839	1805
1342	APCCPPQYNVVQ	1.3985	1.2385	-0.1600	1319
1377	KAGSPPQYLLRY	0.8501	0.9785	0.1283	1746
1413	FGCIPPRYKLCV	1.2356	1.3358	0.1002	1714
1418	ERSHPPRYRGIG	1.9136	1.9722	0.0586	1661
1431	SARRPPRYLLSA	1.3053	1.5394	0.2341	1852
1435	KPGSPPRYLLRY	1.7346	1.5438	-0.1908	1273
1441	RSTKPPRYKCGI	1.9945	1.8601	-0.1344	1370
1442	RSPIPPRYLCSL	1.6750	1.5751	-0.0998	1425
1447	RFQEPPRYRDIC	2.0219	2.1150	0.0931	1707
1448	RELSPPRYKLVC	1.9797	2.0790	0.0993	1712
1454	AVLCPPRYPKLA	2.1943	2.0569	-0.1373	1362
1460	AAGPPPRYCSST	3.1848	2.9543	-0.2305	1192
1461	CATRPPRYSSGW	3.0693	2.8816	-0.1878	1277
1463	TGNSPPRYRLLM	0.9550	1.0465	0.0915	1705
1471	YGSSPPRYTFAA	1.6591	1.5709	-0.0882	1451
1486	LATQPPRYKYTP	1.7710	1.6593	-0.1117	1409
1487	CLPCPPRYRGNQ	2.6210	2.7115	0.0906	1703
1488	LHPHPPRYLLLS	1.5063	1.7093	0.2030	1824
1491	RLPPPPRYTVPR	2.4490	2.3085	-0.1406	1357
1495	VLPLPPRYRFRD	2.1534	1.9602	-0.1932	1262
1496	LQPPPPRYKRFS	2.5752	2.5864	0.0112	1602
1497	RPPIPPRYRRYA	2.6495	2.7548	0.1053	1719
1519	QLRFPPSYRNRT	1.5801	1.5134	-0.0667	1483
1521	RRAQPPSYMHIF	1.9146	2.0382	0.1236	1741
1523	RCSLPPSYCSKL	2.4748	2.7121	0.2373	1857
1524	LGTTPPSYKVRW	1.6087	1.5574	-0.0513	1510
1526	VHRIPPSYVKMI	1.6474	1.8731	0.2257	1839
1528	PCLSPPSYTFPA	2.2456	2.1503	-0.0953	1433
1529	KARHPPSYVVYG	1.8540	1.7544	-0.0996	1426
1540	RPLKPPSYSSHQ	0.9300	0.9817	0.0517	1650
1548	CSSCPPSYQGSC	0.9388	0.8570	-0.0818	1459
1551	CARHPPSYCGGD	1.4319	1.3327	-0.0992	1429
1554	AQATPPSYRCCS	2.0692	1.8561	-0.2131	1226
1556	TQSLPPSYHSCR	2.1099	2.1369	0.0270	1627
1558	CLRYPPSYKALL	1.8823	1.9630	0.0806	1690
1562	CCDHPPSYPLHF	1.3695	1.1253	-0.2442	1154

1565	FGYRPPSYSTAY	1.0504	1.1348	0.0845	1695
1626	SHPLPPSYRKFL	1.4332	1.4776	0.0443	1645
1633	RRFSPPSYSKLT	2.2731	2.0770	-0.1960	1257
1634	RRFSPPSYSKLT	2.1041	2.0349	-0.0692	1476
1679	YPSYPPSYPGTW	0.9885	0.9268	-0.0616	1491
1691	RYGPPPSYPNLK	2.4978	2.2485	-0.2493	1136
1727	SGWQPPTYHSGR	1.5225	1.7671	0.2446	1867
1737	CYSTPPTYFELP	1.0393	0.8885	-0.1509	1339
1744	PCLSPPTYTTFQ	2.2941	2.3712	0.0771	1687
1749	RYSDPPTYCLPP	0.3099	0.3912	0.0813	1691
1769	RSPLPPTYLRPF	1.3843	1.4697	0.0854	1697
1773	RVYQPPTYNRII	1.0985	1.0002	-0.0983	1430
1778	CGLIPPTYRGVW	2.4652	2.2284	-0.2367	1169
1806	YSVPPPVYGCHT	1.9514	1.7529	-0.1986	1252
1849	RNYLPPVYSQRG	1.3437	1.4193	0.0756	1684
1889	RRRFPPYYMRRP	1.5172	1.7063	0.1891	1811
1893	CQHRPPYYGYVK	2.5398	2.7879	0.2481	1868
1896	KTFRPPYYHRNC	2.1804	1.9766	-0.2037	1244
1909	PQRQPPYYSCQC	1.4293	1.3804	-0.0488	1512
1925	AYAPPPYYCSNI	0.7414	0.7808	0.0394	1641
1964	YCASPPYYYDSS	1.2512	1.1997	-0.0515	1509
1965	CARVPPYYYDSS	2.2009	2.0803	-0.1206	1391
Class 3	**Y65C mutant reactivity is greater than wt**				
SPOT#	**sequence**	**wt reactivity**	**mut reactivity**	**log ratio**	**ranking score**
19	ASLCLPRYSFRR	1.0750	1.5744	0.4994	1972
20	ASECNPKYSFRR	2.2143	2.4970	0.2828	1887
21	FHHCHPKYSFRR	2.6667	2.9270	0.2604	1874
27	FLLKLARHSKFG	-1.3762	-0.7086	0.6676	1986
44	SAQRSPRALFCL	-0.2453	0.6606	0.9059	1994
47	RREPRPPRAFCL	1.8190	2.4809	0.6619	1985
111	CVKEPPAYTFRD	0.6917	1.0921	0.4004	1950
112	CWREPPAYTYRD	1.3955	1.7536	0.3581	1936
113	CWKEPPAYTFRD	1.4240	1.8236	0.3996	1949
137	CARVPPAYYDFW	0.3116	0.8852	0.5736	1980
192	SPHLPPCYQHFN	1.6007	2.0115	0.4108	1953
242	IRLLPPDYKIQC	0.2942	0.9817	0.6875	1988
405	KKCFPPFYPSPT	1.7899	2.3292	0.5393	1976
442	LCGYPPFYSKHH	1.2482	1.8506	0.6024	1981
443	LCGYPPFYSNHG	1.1214	1.6851	0.5637	1978
444	LCGFPPFYSNTG	0.4930	1.1159	0.6229	1982
562	PCWFPPGYPETK	0.3549	0.6337	0.2787	1883
563	PCWFPPGYPEAK	0.0961	0.5850	0.4889	1969
642	LCGCPPGYYRVG	0.6344	1.0303	0.3959	1947
643	LCGCPPGYFRIG	0.8003	1.0907	0.2904	1893
651	NCLCPPGYTGSR	1.2272	1.5436	0.3164	1916
721	KVTLPPHYRYGM	1.5670	1.8938	0.3268	1919
722	LVNMPPHYRYLC	0.8675	1.1957	0.3282	1921
733	KRFTPPIYQPKF	1.6847	1.9363	0.2516	1871
743	CTSKPPIYLQHQ	2.0199	2.3614	0.3415	1929
747	IYRKPPIYKQHV	2.0337	2.3255	0.2919	1895
748	IYRKPPIYKQHR	2.2479	2.5280	0.2800	1885
756	PCLSPPIYTTFQ	0.9647	1.3951	0.4305	1958
785	CMAVPPKYRHMQ	2.6060	3.0319	0.4259	1955
825	SVCIPPKYGYLG	1.4262	1.7667	0.3405	1928

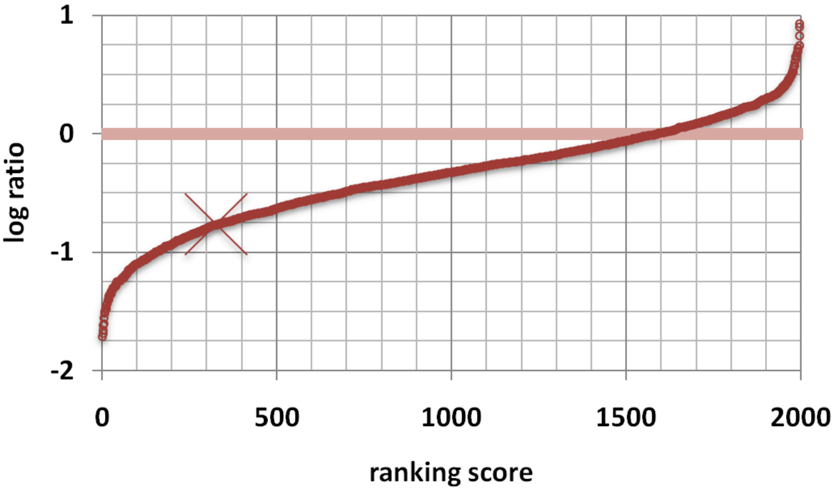
831	YRSTPPLYRHAA	1.8303	2.4020	0.5717	1979
858	YCAKPPLYNWND	1.8781	2.3700	0.4919	1971
919	RNLPPPLYYRPV	0.9184	1.5689	0.6505	1984
971	YCTLPPMYPGSS	1.4188	1.9428	0.5239	1975
979	YNNEPPMYRKGT	0.9346	1.6188	0.6841	1987
1015	YGMVPPNYCSGP	0.6408	1.4722	0.8314	1993
1075	LLCTPPPYAHGR	1.6828	2.2245	0.5417	1977
1102	LYGCPPPYHTFK	1.9172	2.1959	0.2788	1884
1113	SRREPPPYGYRK	1.8677	2.1556	0.2879	1891
1468	RLWRPPRYPRKT	0.8472	1.1462	0.2990	1899
1525	KSSGPPSYFIKR	1.6620	1.9123	0.2503	1869
1550	CSSCPPSYGGSC	1.0701	1.3718	0.3017	1903
1563	CGTYPPSYNLTF	1.5994	1.9695	0.3701	1939
1632	RKLSPPSYCKLA	2.1063	2.3838	0.2774	1882
1801	CFQYPPVYVGKI	0.4449	0.7294	0.2845	1888
1804	APRRPPVYYKFI	1.4155	1.8425	0.4270	1956
1910	ITQSPPYYRCVC	0.9244	1.2341	0.3098	1908

The relative intensities of wt and the Y65C WW domain binding to SPOT peptide arrays was assesed as follows: To avoid as much saturated array elements as possible, the lower quartile cutting value of pixel intensity was chosen to calculate signal intensity (SI) using an image analysis software (genespotter v2.6.0, MicroDiscovery GmbH). To allow a robust comparison, SI was scaled by log-2 and centered by substracting the log-2 product of the median value over both datasets. The resulting transformation is refered to as the reactivity (see Figures below). The effect of the mutation was calculated by substracting the wt reactivity from the mutant reactivity. The resulting log ratio was used to classify peptides in terms of the relative reactivity. Log ratio values lower than -0.2500 classified peptides with mutant reactivity significantly lower than wild-type. This class comprises 1116 out of total 1958 peptides. Inversely, log ratio values greater than 0.2500 classified peptides with mutant reactivity significantly greater than wt. This class comprises 123 out total 1958 peptides. Data was ranked from the lowest log ratio value (-1.7) to the highest one (0.9). (See Table 3 and Figures below).

The data shown in figures below was filtered to eliminate data from spots where the image analysis software reported no signals on both datasets. Class 1: A list of 288 peptides for which the value of ‚no signal' is false and log ratio < -0.2500 is true . Class 2: A list of 161 peptides for which the value of ‚no signal' is false and -0.2501 > log ratio > 0.2501 is true. Class 3: A list of 47 peptides for which the value of ‚no signal' is false and log ratio > 0.2500 is true.



Scatter plot of Y65C mutant WW domain reactivity as a function of wild-type reactivity. The datasets show a good correlation (R^2= 0.8767) but the slope shows a significant departure from 1 (slope= 0.8641). This is in agreement with the observation that the binding profile of both constructs of the WW domain are generally similar, but that the mutant reactivity is compromised. The x-cross shows the data point corresponding to the peptide SPPGPPPYGKRA (SPOT#: 1221). Strong reactivities for this peptide were recorded in both datasets.



Distribution of the calculated log ratio (log ratio = mut reactivity - wt reactivity). This parameter indicates the effect of the mutation with negative values corresponding to binding events where mutant reactivity is less than the wild-type (suggesting a loss of function) and with positive values corresponding to the Y65C mutant reactivity greater than that of the wild-type. The x-cross shows the data point corresponding to the peptide SPPGPPPYGKRA (SPOT#: 1221). A log ratio value of -0.7664 indicates a considerably compromised binding behaviour of the Y65C mutant PQBP1-WW to this selected peptide.