Supplemental Table S2

Table S2. Mutant and Wild Type TWOW-1 Binding Arranged by Binding Levels

A	B	C	D	E	F	G	H	I	J	K	L	M	N
mys allele	βPS AA Change	β3 AA	Domain	Relative TWOW-1 Binding		Relative TWOW-1 Binding	WT vs Mut	Relative noMn/wMn	Relative noMn/wMn		Relative PS2 Integrin Level		Int. with rhea
				Median	S.E.	Z score	p=	Z score	Median	S.E.	Median	S.E.	
G4	S196F	S123	MIDAS	0.00	0.01	-12.6	0.000	-9.1	0.00	0.09	0.83	0.08	G4
b47	A293T	A225	MIDAS/SyMBS	0.20	0.24	-10.4	0.000	-6.2	0.38	0.38	0.09	0.00	b47
b69*	S272+SVRQ	N204+	MIDAS/SyMBS	0.28	0.15	-9.5	0.000	-5.7	0.44	0.20	0.29	0.04	b69
b13*	E274V	E206	MIDAS/SyMBS	0.29	0.03	-9.4	0.000	-6.0	0.40	0.09	0.36	0.06	b13
b26*	G227S	G154	SyMBS	0.35	0.11	-8.6	0.000	-6.4	0.35	0.14	0.34	0.04	b26
b33	I50F	L17	PSI	0.88	0.13	-2.7	0.149	0.7	1.29	0.10	0.63	0.08	b33
b66	P336S	P268	β-I	0.92	0.10	-2.3	0.180	-1.2	1.03	0.06	0.63	0.09	b66
G1	delT825*>	delT741>	Cytoplasmic	0.96	0.12	-1.9	0.503	1.46	1.39	0.07	0.68	0.10	G1
b65*	A310T	A242	β-I	0.98	0.22	-1.6	0.610	2.8	1.56	0.28	0.53	0.13	b65
b20*	P242L	P170	β-I	1.02	0.06	-1.2	0.295	-2.3	0.89	0.07	0.56	0.07	b20
WTm5				1.02	0.32	-1.1	0.220	0.9	1.32	0.49	0.38	0.07	
b70*	S836T, P841T	S752,I757	Cytoplasmic	1.02	0.14	-1.1	0.390	0.3	1.24	0.11	0.61	0.23	b70
b48*	A325T	A257	β-I	1.04	0.15	-0.9	0.432	-0.5	1.13	0.09	0.72	0.08	b48
b53*	E387V	N319	β-I	1.05	0.08	-0.8	0.205	-2.1	0.91	0.10	0.40	0.05	b53
WTm8				1.06	0.09	-0.7	0.776	-1.2	1.04	0.16	0.74	0.09	
WTm1				1.06	0.05	-0.7	0.440	-0.5	1.13	0.04	0.74	0.11	
b42	G347S	N279	β-I	1.09	0.15	-0.3	0.831	1.0	1.33	0.18	0.45	0.02	b42
WTm2				1.09	0.07	-0.3	0.616	-0.3	1.16	0.05	0.74	0.14	
ts2	G347D	N279	β-I	1.10	0.11	-0.2	0.595	1.4	1.38	0.12	0.44	0.05	ts2
WTm9				1.10	0.18	-0.2	0.296	1.7	1.41	0.23	1.06	0.16	
b4	C701Y	C617	β-TD	1.11	0.04	-0.1	0.634	-0.3	1.16	0.04	0.70	0.07	b4
WTm6				1.11	0.00	-0.1	0.788	-0.6	1.12	0.06	0.88	0.08	
WTm3				1.12	0.04	-0.1	0.805	0.3	1.24	0.05	0.52	0.11	
b34	G707S	G623	β-TD	1.12	0.07	0.0	0.451	-0.2	1.17	0.07	0.96	0.07	b34
WTm4				1.14	0.00	0.2	0.905	0.0	1.19	0.00	0.50	0.03	
WTm7				1.17	0.06	0.6	0.607	1.0	1.33	0.05	0.64	0.05	
b32	V763I	S677	β-TD	1.21	0.11	0.9	0.962	2.1	1.46	0.10	0.51	0.06	b32
b24*	E600K	E522	I-EGF2-3	1.21	0.1	1.0	0.102	-0.4	1.14	0.06	0.61	0.08	b24
b39*	G679D	G595	I-EGF4	1.23	0.08	1.2	0.311	1.1	1.33	0.15	1.03	0.27	b39
b7	D404N	D336	β-I	1.26	0.15	1.5	0.586	0.4	1.25	0.21	0.61	0.08	b7
b57*	G339S	G271	β-I	1.28	0.14	1.8	0.106	-1.3	0.81	0.01	0.33	0.05	b57
b46*	G596S	G518	I-EGF2	1.34	0.08	2.4	0.001	0.7	1.29	0.09	0.55	0.09	b46
Wtm10				1.34	0.05	2.5	0.197	-1.5	1.00	0.09	0.38	0.04	
b1*	G792D	G708	Transmembrane	1.44	0.07	3.5	0.001	-0.9	1.08	0.04	0.50	0.06	b1
b44*	I375F	I307	ADMIDAS	1.44	0.09	3.6	0.001	3.2	1.61	0.13	0.38	0.12	b44
b49*	H342Y	H274	β-I	1.45	0.10	3.6	0.003	1.1	1.34	0.11	0.54	0.04	b49
b40*	V775D	P691	β-TD	1.51	0.08	4.4	0.001	-0.2	1.16	0.09	0.56	0.08	b40
b63*	G531D	G458	I-EGF1	1.53	0.08	4.6	0.001	2.0	1.46	0.10	0.39	0.06	b63
b30*	I298F	T230	β-I	1.56	0.10	4.9	0.001	0.4	1.25	0.09	0.24	0.03	b30
b45*	R312Q	H244	ADMIDAS	1.57	0.19	5.0	0.000	3.2	1.62	0.27	0.29	0.09	b45
b55	R587Q	S511	I-EGF2	1.58	0.10	5.2	0.000	5.9	1.96	0.10	0.91	0.08	b55
b50	S272+Q	N204+	MIDAS/SyMBS	1.65	0.06	5.9	0.000	4.2	1.75	0.13	0.53	0.05	b50
b22*	R676C	G592	I-EGF4	1.65	0.12	5.9	0.000	4.2	1.74	0.14	0.34	0.03	b22
b3*	C58S	C26	PSI	1.72	0.52	6.7	0.000	3.6	1.66	0.18	0.30	0.05	b3
b38*	L224F	I151	ADMIDAS	1.73	0.33	6.9	0.000	4.5	1.79	0.40	0.69	0.08	b38
b31	G541S	G468	I-EGF1	1.76	0.10	7.2	0.000	8.1	2.25	0.11	1.03	0.07	b31
b56	G395S	G327	ADMIDAS	1.81	0.11	7.7	0.000	5.4	1.90	0.06	0.50	0.08	b56
b37*	C441Y	C374	Hybrid	1.81	0.24	7.8	0.000	-1.4	0.97	0.14	0.23	0.06	b37
b68	C58Y	C26	PSI	1.82	0.11	7.9	0.000	10.1	2.52	0.08	0.44	0.04	b68
b21*	L264F	L196	ADMIDAS	1.84	0.30	8.1	0.000	6.2	2.01	0.54	0.32	0.03	b21
b41	C40Y	C5	PSI	1.90	0.03	8.7	0.000	12.2	2.80	0.12	0.48	0.04	b41
b25	S317L	T249	ADMIDAS	1.91	0.12	8.8	0.000	7.8	2.22	0.09	0.49	0.07	b25
b52	G596R	G518	I-EGF2	1.92	0.24	9.0	0.000	6.8	2.09	0.38	0.36	0.04	b52
XN101	C629S	C549	I-EGF3	1.94	0.46	9.1	0.000	6.3	2.02	0.50	0.05	0.00	XN101
b29	F743I	Y657	β-TD	2.00	0.23	9.9	0.000	4.7	1.81	0.23	0.57	0.03	b29
b58	V409D	L341	ADMIDAS	2.07	0.14	10.6	0.000	9.3	2.41	0.17	0.30	0.02	b58
b67*	D200N	D127	ADMIDAS	2.15	0.11	11.6	0.000	1.5	1.39	0.04	0.25	0.06	b67
b27	V423E	V355	Hybrid	2.21	0.16	12.3	0.000	7.0	2.11	0.24	0.77	0.06	b27
b64	C544Y	C471	I-EGF1	2.40	0.26	14.4	0.000	9.1	2.38	0.29	0.48	0.06	b64
b51	E607K	V529	I-EGF3	3.51	0.17	26.8	0.000	22.3	4.12	0.59	0.35	0.04	b51
b23*	N407Y	N339	ADMIDAS	3.61	0.24	28.0	0.000	14.6	3.11	0.21	0.24	0.03	b23

Table S2. Distribution of TWOW-1 binding of wild type and mutant βPS integrins expressed together with αPS2C in stably transformed cell lines. Data are sorted by increasing binding levels. Also shown is the ratio of binding without Mn^{2+} (non-activating conditions) to binding with Mn^{2+}(activating conditions) and expression levels. Gray shading in all Columns indicates wild type integrins.

Columns are:

A. Mutant allele name. * multiple independent cell lines were tested. Shading of boxes indicates effects of the mutation on TWOW-1 binding levels; red, z scores less than -10; dark pink, z scores between -5 and -10; no fill, z scores between -2.7 and 2.4; light green, z scores between 2.4 and 4.9; dark green z scores between 5 and 10; blue, z scores greater than 10.

B. Amino acid change in βPS integrin. S272+SVRQ and S272+Q (b69 and b50) indicates insertion of VRQ or Q following amino acid 272. delT825*> (*mys*^{G1}) refers to carboxy-terminal, 21 amino acids deleted and replaced by 25 essentially random amino acids (due to a splice site mutation).

C. Corresponding amino acid in human β3 integrin.

D. The domain of the β subunit in which the mutation is located or is predicted to alter.

E. The median of the PS2C integrin staining for 3-12 experiments, normalized to the WT1 cell line (untagged wild type line 1).

F. S.E. of Column E.

G. The z score, number of standard deviations below (negative values) or above (positive values) the mean TWOW-1 binding of the 10 independent lines expressing wild type PS2C integrins. See Column A for shading description.

H. P value determined by Student's t-test comparison of mutant TWOW-1 binding with the tests of the 10 independent lines expressing wild type PS2C integrins. Significant values for reduction in binding are shaded in pink and for increased binding in light green.

I. The z-scores of the "activation index" (see Column J) -- number of standard deviations below (negative values) or above (positive values) the mean of the 10 independent lines expressing wild type PS2C integrins. See Column A for shading description.

J. The median of the "activation index" [ratio of TWOW-1 binding without Mn^{2+} (non-activating conditions) to binding with Mn^{2+}(activating conditions)]. Each experiment was normalized to the "activation index" of WT1 cell line (included in all experiments).

K. S.E. of Column J.

L. Median of PS2C integrin expression levels as determined by staining PS2 integrins with an αPS2 antibody and normalizing levels to the WT1 cell line. Red shading indicates expression levels more than 2 standard deviations below the mean of the 10 independent lines expressing wild type PS2C integrins.

M. S.E. of Column 9.

N. Results of genetic interaction tests with *rhea*, which encodes talin (from supplemental Table S5). Dark pink indicates an enhancing interaction—flies with the βPS mutation are more lethal with only one wild type copy of *rhea* than when they contain 2 wild type copies of *rhea*. Green indicates a rescuing interaction-- flies with the βPS mutation are less lethal with only one wild type copy of *rhea* than when they contain 2 wild type copies of *rhea*. No shading indicates a lack of significant difference in lethality. Tan shading indicates that there was insufficient lethality at high temperatures to perform the test. Some mutants were completely lethal (brown) at even the lowest temperature tested and so the interaction test could not be performed.