## **Supplemental Table S5**

Α	В	С	D	E	F	G	н	1	J	К	L	M	N	0
		Test#1		Test#2		Test#2		_	_	_	_	rhea/+	+/+	
mys	mys	Viability		males		females		Progeny	Progeny	Progeny	Progeny	% viability	% viability	-
TWOW	rhea int.	male vs female	_	mys/wt	<u> </u>	mys het./wt		males	males	females	females	males/females	males/females	Temp
Shading	Shading	Column M/N	P=	(I/J)/(mys+I/J)	P=	(K/L)/(mys+K/L)	P=	mys; rhea/+ rhea/+	mys; +/+	mys/+; rhea/+	mys/+; +/+	(Column I/K)	(Column J/L)	°C
547	b47	0.3	0.00	0.2	0.00	0.9	0.56	7	26	129	141	5%	18%	22
b69	b69	0.1	0.00	0.1	0.00	0.7	0.06	2	24	60	75	3%	32%	25
513	b13	0.1	0.00	0.1	0.00	1.1	0.71	1	12	44	39	2%	31%	22
526	b26	leth 18				1.1	0.56	0	0	164	134	0%	0%	18
<b>b</b> 33	b33	2.3	0.01	2.2	0.01	0.9	0.59	40	16	170	153	24%	10%	31*
<b>b66</b>	b66	9.0	0.01	8.9	0.01	1.0	0.91	10	1	366	328	3%	0%	18
<b>b</b> 65	b65	1.7	0.01	1.4	0.10	0.8	0.12	87	54	106	114	82%	47%	31*
20	b20	0.8	0.48	0.6	0.02	0.7	0.02	51	75	81	101	63%	74%	31*
o70	b70	0.1	0.00	0.1	0.00	0.8	0.34	4	62	226	261	2%	24%	22
048 053	b48 b53	0.6	0.02	0.5	0.00	0.8	1.6	37	69 46	103	107 105	36%	64% 44%	31* 31*
053 042	b53 b42	0.7	0.22	0.3	0.00	0.4	0.00	113	129	55 164	105	29% 69%	76%	31*
s2	ts2	0.9	0.34	1.2	0.13	1.8	0.00	71	47	281	153	25%	31%	28
52 04	b4	0.5	0.00	0.3	0.00	0.6	0.00	20	57	95	133	21%	44%	31*
034	b34	1.6	0.05	1.6	0.05	0.9	0.62	78	44	88	80	89%	55%	31*
032	b32	1.8	0.03	1.7	0.05	1.1	0.75	63	31	65	59	97%	53%	22
024	b24	2.2	0.00	2.2	0.00	1.0	0.90	87	34	106	90	82%	38%	31*
039	b39	1.2	0.37	1.3	0.32	1.0	0.78	63	44	94	82	67%	54%	31*
57	b7	1.06	0.69	1.0	0.91	0.9	0.33	149	133	232	220	64%	60%	31*
57	b57	24.9	0.00	23.2	0.00	0.9	0.42	53	2	161	151	33%	1%	31*
<b>546</b>	b46	0.9	0.67	1.0	0.90	1.0	1.00	115	103	159	132	72%	78%	31*
<b>51</b>	b1	1.2	0.43	1.3	0.22	1.1	0.59	61	40	386	300	16%	13%	31*
<b>544</b>	b44	2.8	0.00	2.4	0.00	0.9	0.59	132	49	224	235	59%	21%	28
<b>549</b>	b49	1.1	0.83	1.0	0.97	0.9	0.58	43	38	51	48	84%	79%	31*
<b>540</b>	b40	0.6	0.17	0.1	0.00	0.2	0.00	12	77	18	66	67%	117%	31*
<b>b63</b>	b63	0.9	0.67	0.8	0.22	0.8	0.20	119	129	148	149	80%	87%	31*
030	b30	13.3	0.00	15.8	0.00	1.2	0.27	124	7	282	212	44%	3%	18
545	b45	8.2	0.00	5.7	0.00	0.7	0.00	39	6	149	187	26%	3%	31*
55 50	b55 b50	0.4	0.00	0.1	0.00	0.3	0.00	18 57	144 66	44	134 89	41% 56%	107% 74%	31* 31*
000 022	b22	leth18	0.22	0.8	0.20	1.0	0.78	0	00	102	118	0%	0%	18
022 03	b22		0.01	5.9	0.01	1.4	1.00	20	3	130	91	16%	3%	28
53 538	b38	0.8	0.35	0.6	0.01	0.7	0.04	55	82	84	101	65%	81%	31*
b31	b30	1.1	0.56	1.1	0.72	0.9	0.54	149	123	174	158	86%	78%	31*
556	b56	3.7	0.00	3.2	0.00	0.8	0.14	83	23	166	171	50%	13%	31*
o37	b37	0.7	0.12	0.8	0.28	1.1	0.64	52	58	111	85	47%	68%	31*
68	b68	13.9	0.00	11.3	0.00	1.0	0.86	55	4	211	213	26%	2%	22
021	b21	0.7	0.66	0.6	0.56	0.8	0.20	2	3	169	169	1%	2%	31*
<b>5</b> 41	b41	2.3	0.04	2.3	0.03	1.0	0.90	26	10	62	55	42%	18%	18
b25	b25	0.9	0.61	0.9	0.43	0.9	0.46	143	143	193	178	74%	80%	31*
52	b52	3.6	0.04	2.8	0.11	0.8	0.12	10	3	156	169	6%	2%	25*
529	b29	2.2	0.00	2.0	0.01	0.9	0.29	59	26	213	205	28%	13%	31*
558	b58	0.5	0.02	0.5	0.02	0.9	0.68	30	49	110	98	27%	50%	31*
67	b67	0.1	0.00	0.1	0.01	1.2	0.44	1	9	181	143	1%	6%	18
527	b27	3.5	0.00	3.8	0.00	1.0	0.88	73	17	284	231	26%	7%	31*
64	b64	9.5	0.00	8.9	0.00	1.0	0.80	20	2	344	328	6%	1%	18
51	b51	2.1	0.02	2.2	0.03	1.1	0.68	32	13	449	383	7%	3%	18
523 Mild two	b23 e controls	leth18				0.9	0.49	0	0	88	93	0%	0%	18
ind typ	mys+		0.90			1		109	97	92	84	118%	115%	18
	mys+	1.19	0.43			1	$\vdash$	91	75	88	86	103%	87%	22
	mys+	1.00	1.00				+	131	109	131	109	100%	100%	25
	mys+	1.00	0.15			1	+	157	120	165	160	95%	75%	28
	mys+	0.95	0.72				+ +	154	135	230	191	67%	71%	31*

<u>Table S5.</u> Genetic interactions between *mys* mutants and *rhea*. Mutant *mys* alleles were tested for genetic interactions with *rhea* (encoding talin) that alter lethality. *mys*<sup>+</sup>; *rhea*<sup>2</sup>/*rhea*<sup>+</sup> males were mated to *mys/mys*; *rhea*<sup>+</sup>/*rhea*<sup>+</sup> females. Numbers of male and female progeny of each class is given in Columns I-L. The temperature of the crosses that result in significant lethality varied for different *mys* alleles and is indicated in Column O. In this Column 31\* indicates that the cross and egg laying were done at either 28°C or 25°C and then shifted up to 31°C. 25\* indicates that egg laying was done at 18°C and then shifted to 25°C.

The genetic interaction results are presented based on the previously determined TWOW-1 binding ability of the *mys* alleles (from low to high) and color coding of the alleles in Column A is the same as in Table S2. Color coding of the results of genetic interactions between *mys* alleles and *rhea* is shown in Column B. Where reduction in talin levels rescues lethality of the *mys* allele shading is light green. Where

reduction in talin levels increases lethality of the *mys* allele shading is magenta. Where reduction in talin levels had no effect no shading is applied.  $mys^{b26}$ ,  $mys^{b22}$ , and  $mys^{b23}$  males were lethal at the lowest temperature tested (18°C) and so could not be examined for *rhea* interactions (brown in Columns B, C, M, and N). Twelve other alleles also could not be examined for interactions with *rhea* in this test because they did not show enough lethality even at the highest temperature (31°C) tested (tan in Columns B, C, M and N).

The relative viability of males hemizygous for the indicated *mys* allele (as *mys* is on the X chromosome) with one versus two wild type copies of *rhea* (located on chromosome 3) was determined in two ways and is displayed in Columns C and E together with P values from two-proportion Z-tests (Columns D and F). Effects of talin reduction on females heterozygous for the *mys* allele is given in Column G (P values in Column H).

For the first two-proportion Z-test, relative lethality was determined by comparing two proportions and the results are given in Column C (with P value in Column D). The first proportion (Column M) is the number of *mys* males with only one wild type copy *rhea* (Column I) as compared to the number of females heterozygous for both *mys* and *rhea* (Column K). The second, control, proportion (Column N) is the number of *mys* males with two wild type copies of the *rhea* (Column J) compared to the number of females heterozygous for *mys* with two wild type copies of rhea (Column L). Dividing the values when only one copy of *rhea* is present (Column M) by that when two copies are present (Column N) results in one test of relative viability (Column C and D). This test has the advantage of having all progeny, experimental and controls, raised in the same vials under identical conditions. It has the caveat, however, that mutant *mys* allele may not always be completely rescued by *mys*<sup>+</sup> in heterozygotes.

The second test of *mys* and *rhea* interaction uses two different proportions. The first is the number of *mys* males with only one wild type copy of *rhea* (Column I) compared to the number of *mys* males with two wild type copies of *rhea* (Column J). The second, control, proportion is from a separate cross. *mys*<sup>+</sup>; *rhea*<sup>2</sup>/*rhea*<sup>+</sup> males were mated to wild type females at all temperatures. Results of this cross are at the end of the table and labeled mys+. In these control crosses, wild type males heterozygous for *rhea* (Column I) were compared with wild type males (Column J). Comparing these two proportions gives a relative viability for males (Columns E and F). The same type of analysis was used to compare females heterozygous for *mys* alleles and those bearing two wild type copies of *mys* (Columns G and H).

For male lethality the results of both analyses are very consistent. 17 of 19 of cases where *rhea*/+ shows rescue of *mys* lethality in males are detected by both tests and the exceptions, *mys*<sup>b65</sup> and *mys*<sup>b52</sup>, trend in that direction in the second test but do not reach significance (P=0.1 & 0.11). Where *rhea*/+ enhances *mys* lethality in males, 9 of 13 are detected by both tests and the exceptions, *mys*<sup>b20</sup>, *mys*<sup>b40</sup>, *mys*<sup>b53</sup>, and *mys*<sup>b38</sup> do not show the interaction in the first test due to *rhea*/+ enhanced lethality in both *mys* males and heterozygous females. Interactions between *rhea*/+ and *mys*/+ in females (magenta and light green in Columns G and H) suggest that these mutant  $\beta$ PS proteins can result in dominant effects in the presence of wild type  $\beta$ PS proteins. This is most prominent in *mys*<sup>b40</sup> and *mys*<sup>b55</sup>.

That heterozygosity for the *rhea*<sup>2</sup> allele has no effect on lethality in the absence of a *mys* mutant allele is shown in the wild type, mys+, crosses (end of table, Columns C and D).