Biology Open

Supplementary Material

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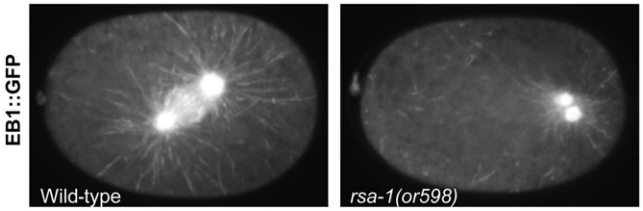


Fig. S1. *rsa-1(or598ts)* has fewer microtubules emanating from the centrosomes. EB1::GFP decorates the growing ends of microtubules. Panels shown are a maximum projection of 25 images acquired as a GFP live stream (300 ms frame rate over 7.5 seconds) of a wild-type and *rsa-1(or598)* embryo expressing EB1::GFP. In wild-type embryos there are more EB1 tracks than in *rsa-1(or598ts)* embryos.

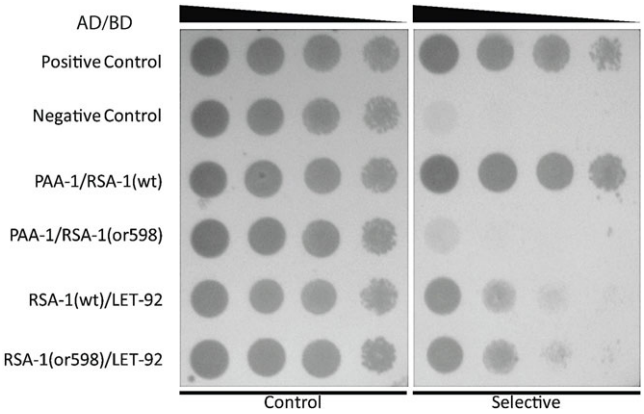
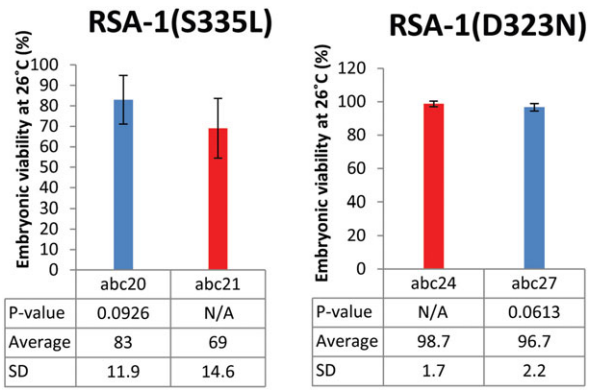


Fig. S2. Yeast-two-hybrid analysis. Control medium is SC−trp−leu to select for the transformed plasmids. Selective medium is SC−trp−leu−his and requires interaction between the activation domain (AD) fusion protein and the binding domain (BD) fusion protein to facilitate growth. The positive control is AD::RAF/BD::RAS and the negative control is AD::Empty/BD::RAS, provided by Addgene. Yeast were plated in a dilution series of 1, 1/10. 1/100, and 1/1000.



	abc20	abc21
P-value	0.0926	N/A
Average	83	69
SD	11.9	14.6

	abc24	abc27
P-value	N/A	0.0613
Average	98.7	96.7
SD	1.7	2.2

Fig. S4. Homozygous embryonic viability at 26˚C of intragenic suppressors with multiple alleles. Red bar indicates reference allele for each amino acid substitution. No statistical difference was observed between the alleles of each suppressor, so only one reference allele was used for subsequent analysis. Error bars represent standard deviation. Embryonic viabilities between alleles were considered to be significantly different if the P-value was less than 0.0500 in an unpaired, two-tailed *t*-test.

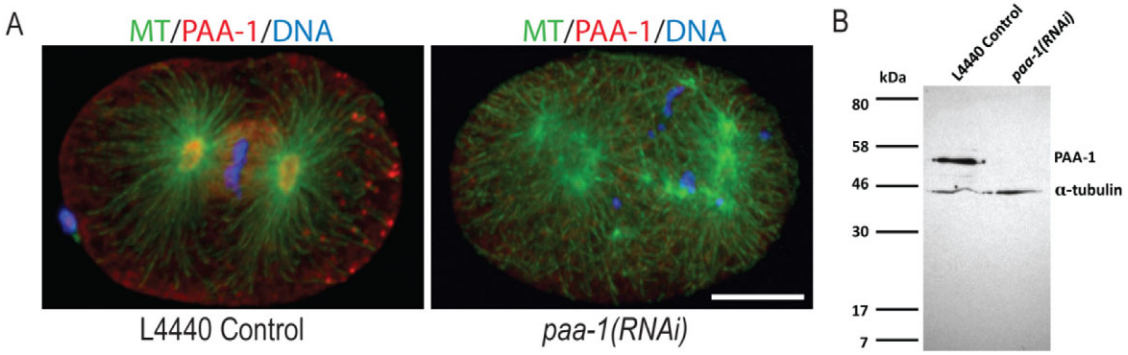


Fig. S3. Confirmation of the specificity of the rabbit-anti-PAA-1 antibody. (**A**) Immunofluorescence staining of *paa-1(RNAi)* embryos after 28 hours of RNAi feeding. Scale bar represents 10 μm. (**B**) Western blot of embryo preparations, from Fig. 1, but with extended field to indicate antibody specificity. PAA-1 migration is consistent with a size of ~55 kDa.

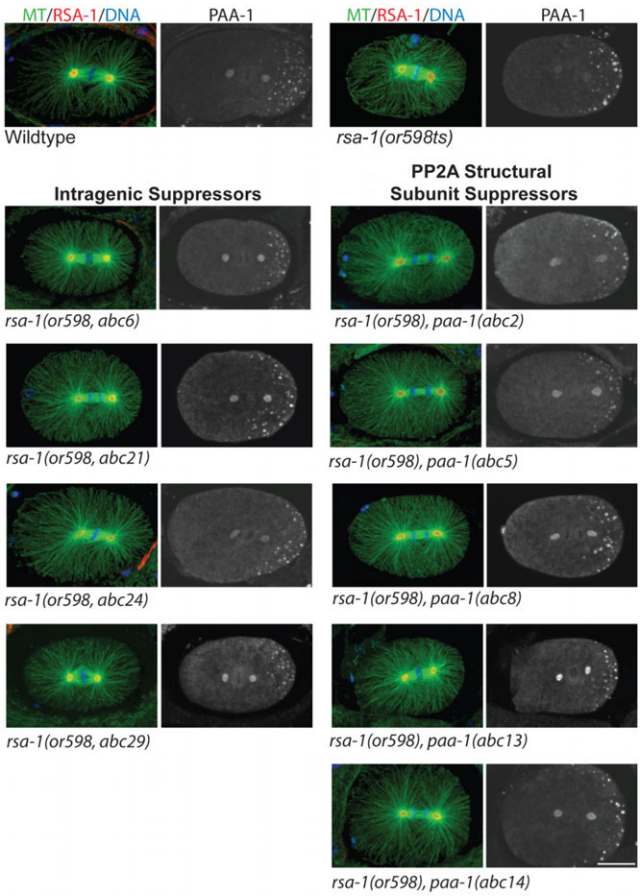
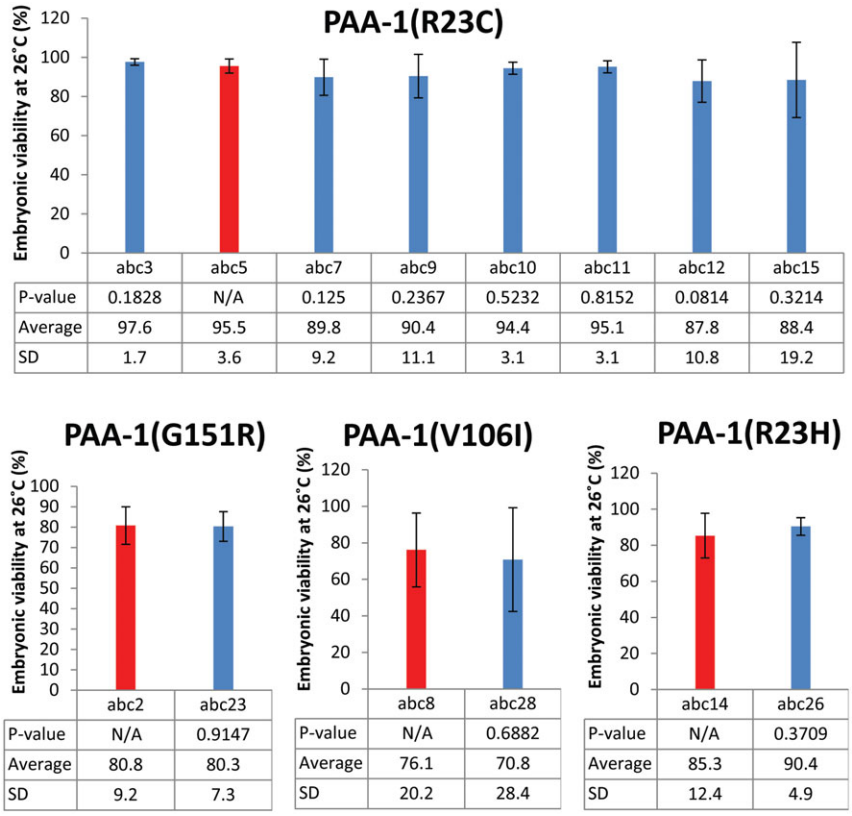


Fig. S5. Interaction suppressors in PP2A/RSA-1 complex restore recruitment of PAA-1 protein to centrosomes. Representative fixed embryos for *rsa-1* and *paa-1* suppressors stained with anti-PAA-1 and anti-RSA-1. Scale bar represents 10 μm.



	abc3	abc5	abc7	abc9	abc10	abc11	abc12	abc15
P-value	0.1828	N/A	0.125	0.2367	0.5232	0.8152	0.0814	0.3214
Average	97.6	95.5	89.8	90.4	94.4	95.1	87.8	88.4
SD	1.7	3.6	9.2	11.1	3.1	3.1	10.8	19.2

	abc2	abc23
P-value	N/A	0.9147
Average	80.8	80.3
SD	9.2	7.3

	abc8	abc28
P-value	N/A	0.6882
Average	76.1	70.8
SD	20.2	28.4

	abc14	abc26
P-value	N/A	0.3709
Average	85.3	90.4
SD	12.4	4.9

Fig. S6. Homozygous embryonic viability at 26℃ of suppressors with multiple alleles in *paa-1*. Red bar indicates reference allele for each amino acid substitution. No statistical difference between multiple alleles of each suppressor so only one reference allele was used for subsequent analysis. Error bars represent standard deviation. Embryonic viabilities between alleles were considered to be significantly different if the P-value was less than 0.0500 in unpaired, two-tailed *t*-test.

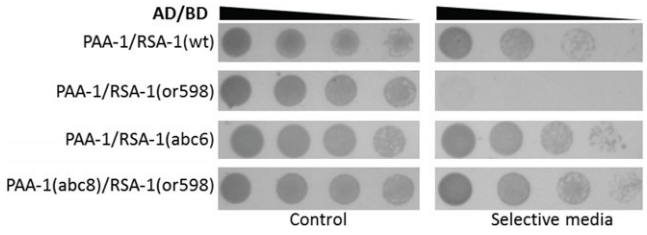


Fig. S7. Yeast-two-hybrid analysis with representative suppressor mutations. Control medium is SC−trp−leu to select for the transformed plasmids. Selective medium is SC−trp−leu−his and requires interaction between the activation domain (AD) fusion protein and the binding domain (BD) fusion protein to facilitate growth. Yeast were plated in a dilution series of 1, 1/10,

Table S1. PCR primers used in this study. Restriction enzyme sites are in bold. Mutagenic lesions are in red.

Primers for yeast two hybrid analysis	
paa-1 into pAct2.2	5′TTT**CCCGGG**CTTGTCGGTTGTCGAAGAAGCC
	5′TTT**CCCGGG**AGGTACAAGCCGAGTGAGTTCTTGG
let-92 into pLexA	5′TTT**CCCGGG**CGCAGGCTTGGCTGCTGCC
	5′TTT**CTGCAG**GGTACAGGAAGTAGTCAGGC
rsa-1 into pLexA/pACT2.2	5′TTT**GGATCC**GCTTGCCAACCGACGAACCTTC
	5′TTT**CTGCAG**GGTATTGCTCGCGTCGTTCG
rsa-1(or598) PCR mutagenesis	5′/Phos/TACCATTTCGAAATTCTGG**G**TCTAAAGGATGATGGACTGTTG
	5′/Phos/CAACAGTCCATCATCCTTTAGA**C**CCAGAATTTCGAAATGGTA
rsa-1(abc6) PCR mutagenesis	5′/Phos/CAAATACCATTTCGAAATTCTG**A**GTCTAAAGGATGATGGACTG
	5′/Phos/CAGTCCATCATCCTTTAGAC**T**CAGAATTTCGAAATGGTATTTG
paa-1(abc8) PCR mutagenesis	5′/Phos/GCCACAGTCGAGGAGACCGTG**A**TCCGTGACAAAGCCGTTGAG
	5′/Phos/CTCAACGGCTTTGTCACGGA**T**CACGGTCTCCTCGACTGTGGC
Primers for PAA-1 antibody production	
paa-1 C-terminus into pGEX-6P-1	5′**CCCGGG**GTACACGAACCTTCTCAAGG
	5′**GCGGCCGC**TTAATGCTTTCTTATAATGCC
paa-1 C-terminus into pMALc5x	5′GGGG**CATATG**TACACGAACCTTCTCAAG
	5′**GCGGCCGC**TTAATGCTTTCTTATAATGCC