

Quantifying western blots: do not let the bias win

By Jiri Veis

from

Egon Ogris lab & antibody facility

The source of non-linearity in Western Blotting:

- 1) Lysate (conc.)***
- 2) Chromatographic effects during PAGE***
- 3) Transfer efficiencies***
- 4) AFFINITY OF ANTIBODIES***
- 5) Detection (signal generation/accumulation)***

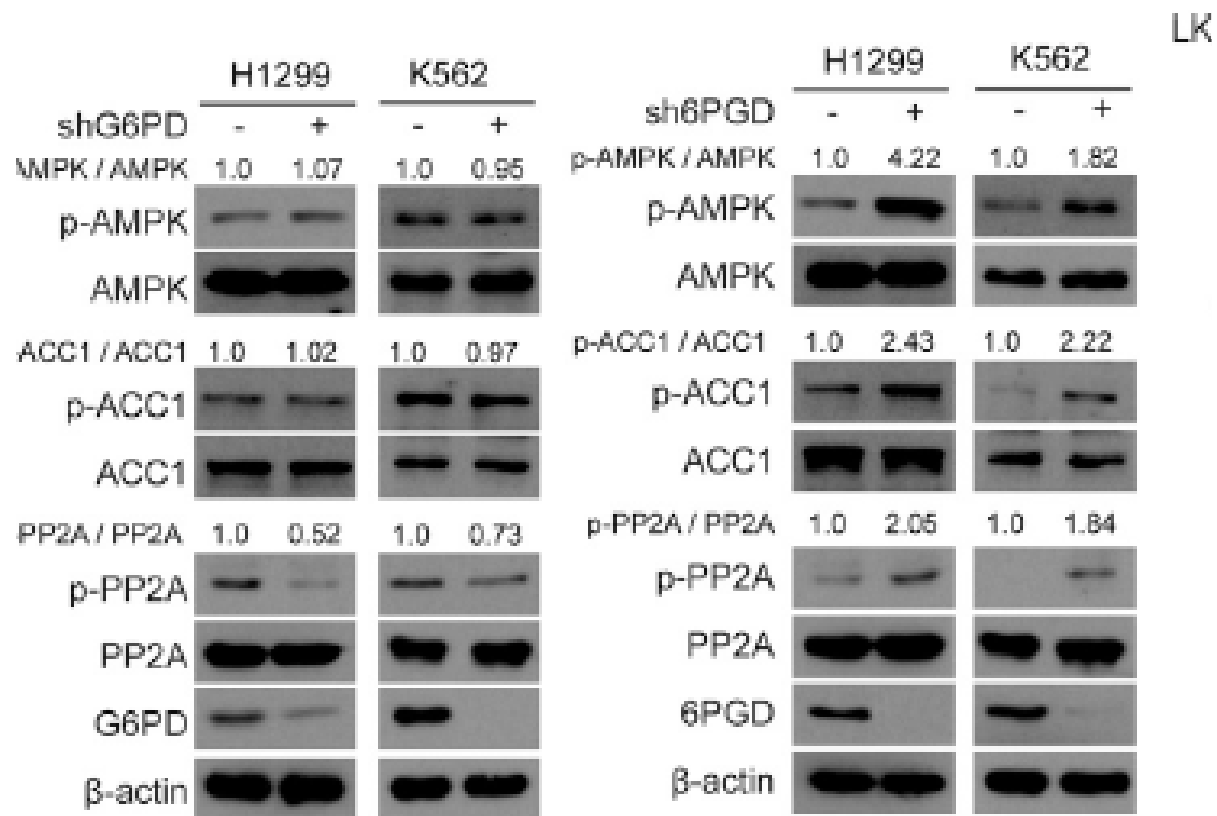
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Licor-Odessey

A bad, yet typical example



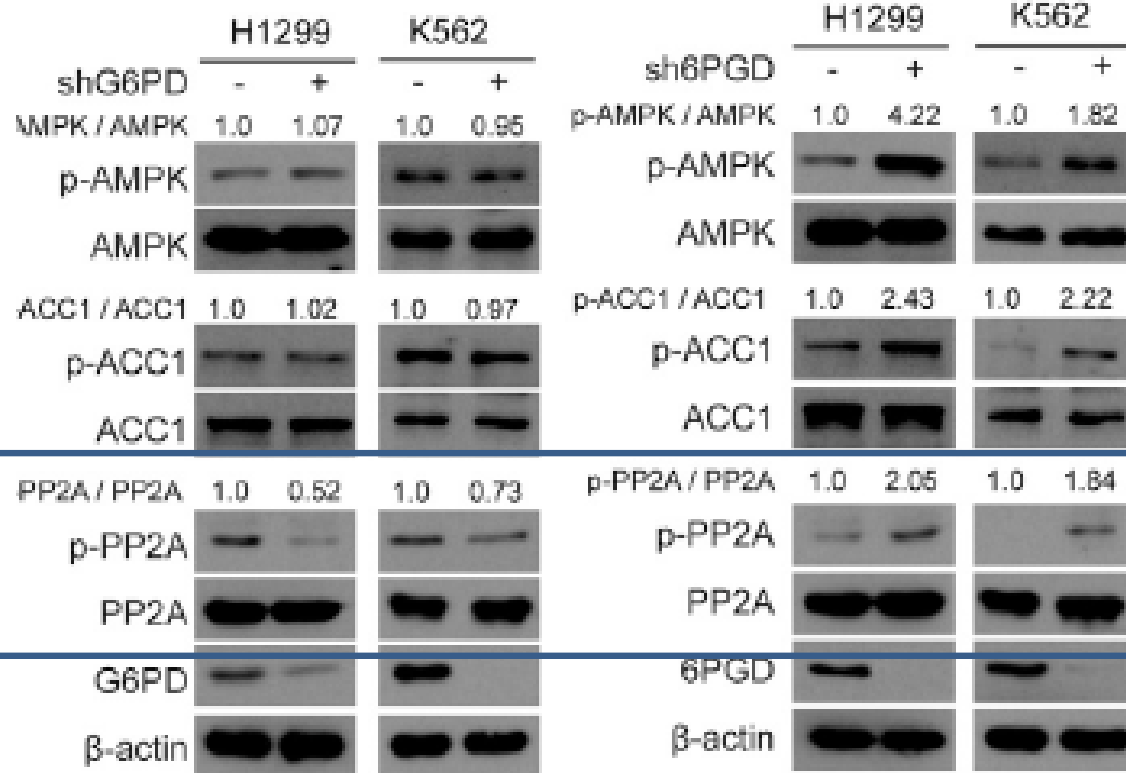
Molecular Cell

Article

γ-6-Phosphogluconolactone, a Byproduct of the Oxidative Pentose Phosphate Pathway, Contributes to AMPK Activation through Inhibition of PP2A

A bad, yet typical example

LK

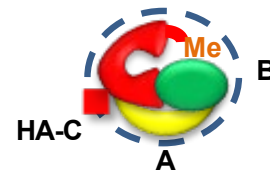
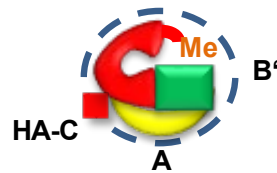
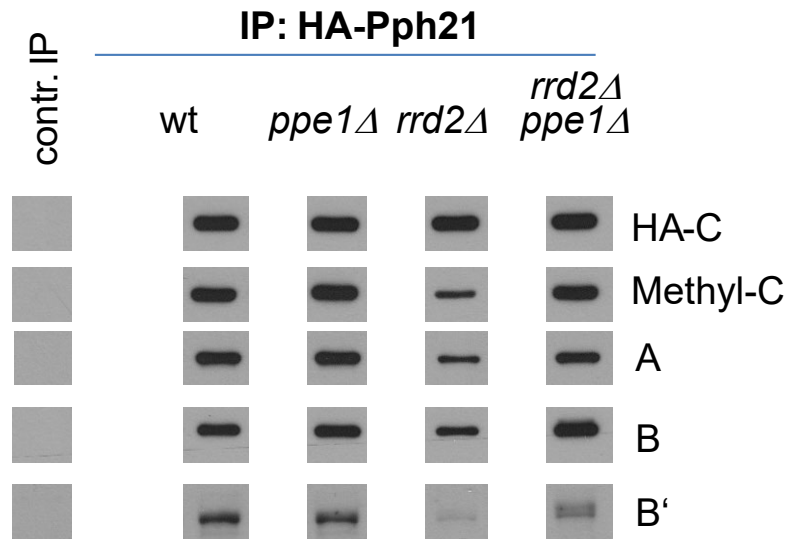


Article

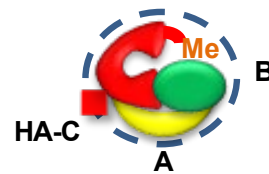
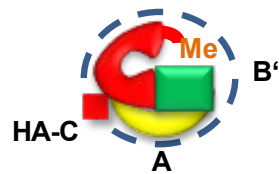
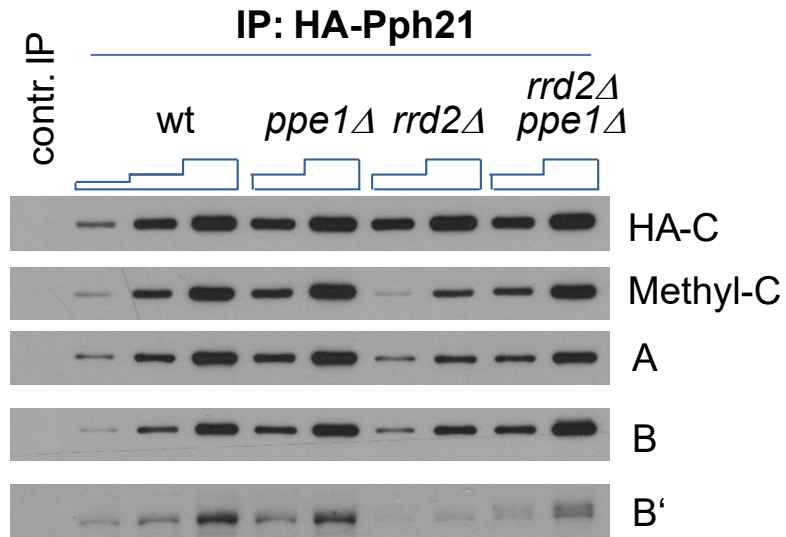
Molecular Cell

γ -6-Phosphogluconolactone, a Byproduct of the Oxidative Pentose Phosphate Pathway, Contributes to AMPK Activation through Inhibition of PP2A

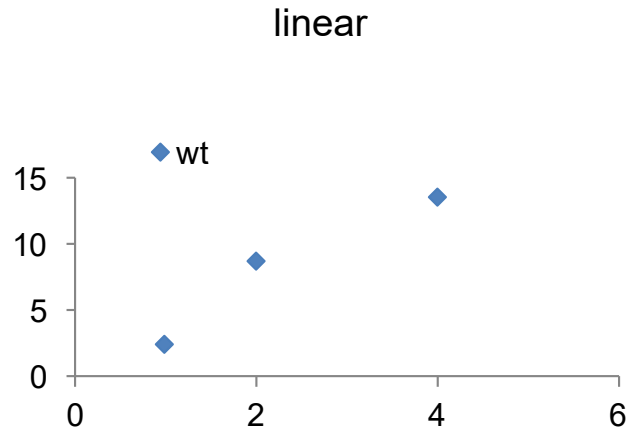
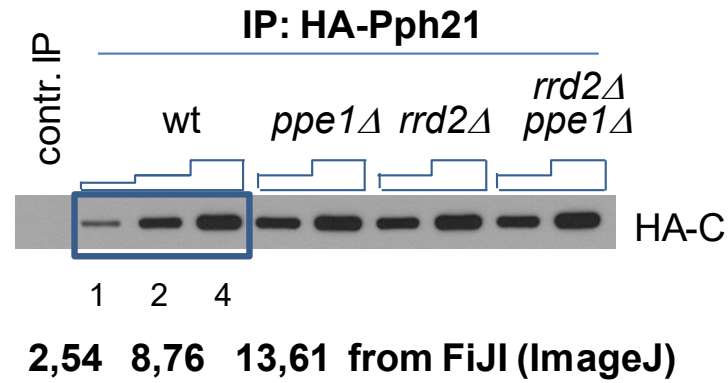
My way to quantify: IP of the PP2A complex

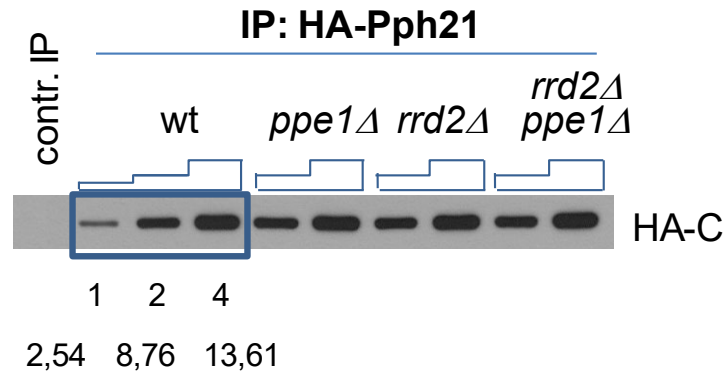


IP of the PP2A complex

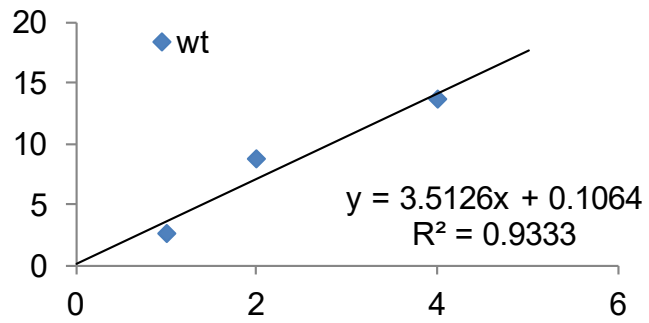


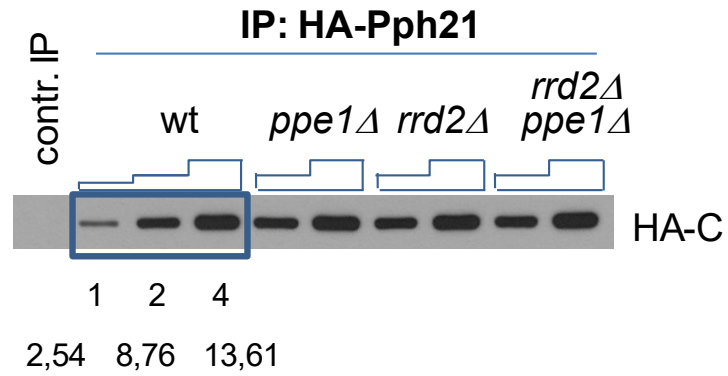
Quantification of Western Blot signals: linear vs logarithmical fit



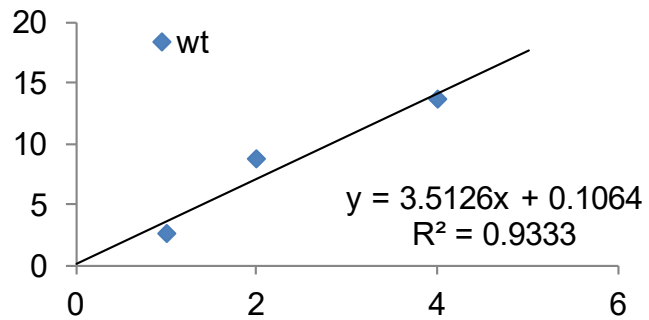


linear

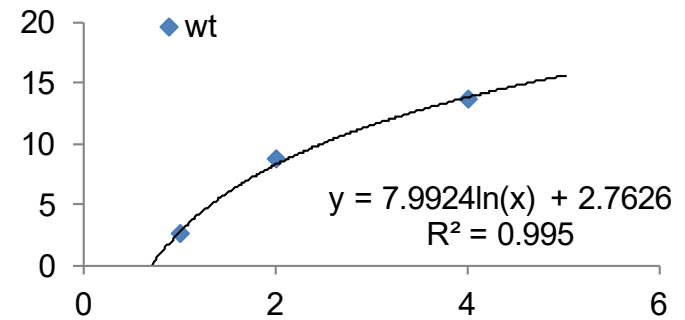


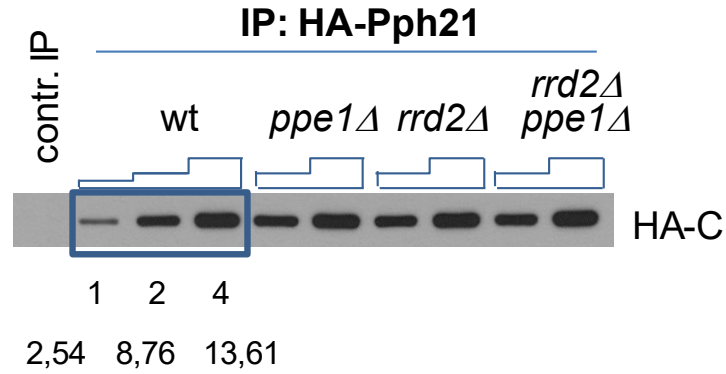


linear

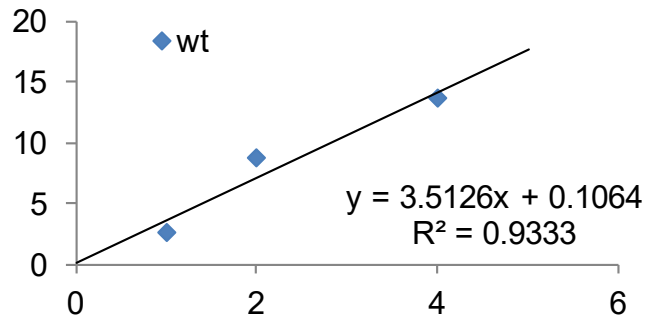


ln

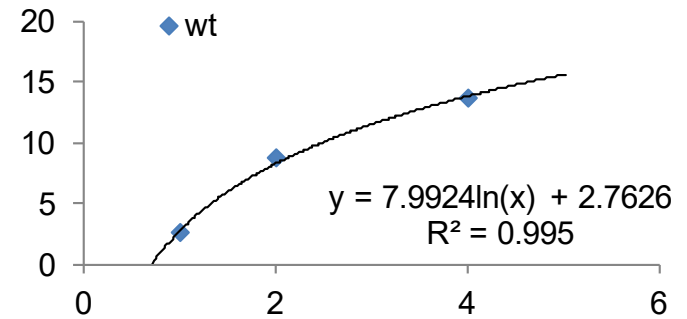




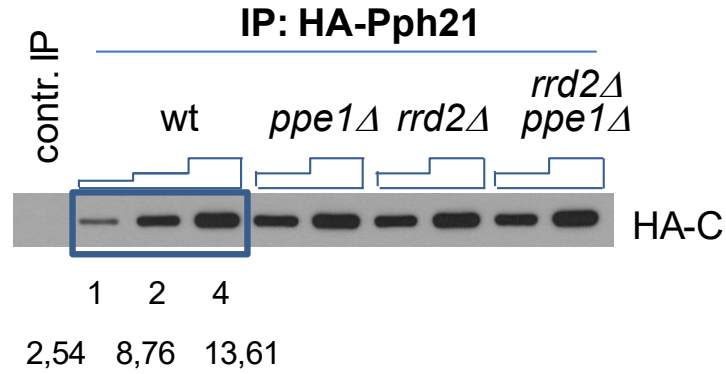
linear



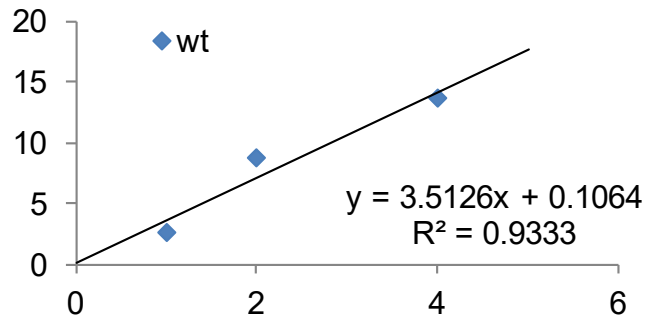
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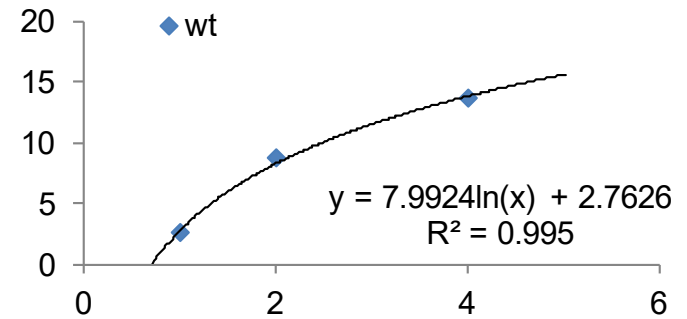
	IP fold	values	ln fit	divided	mean	% wt
wt	1	2,54	0,97	0,97		
	2	8,76	2,12	1,06		
	4	13,61	3,89	0,97	1,00	100,00



linear

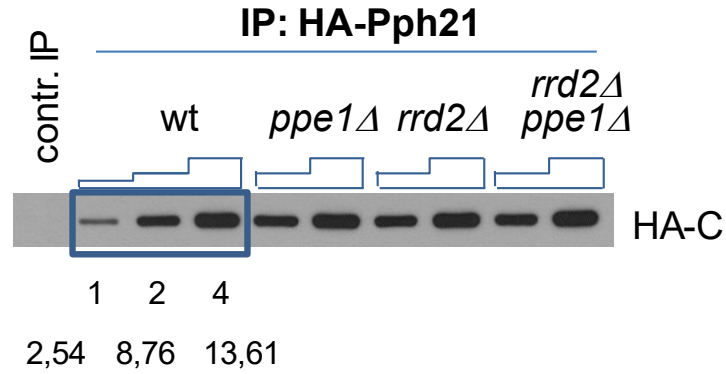


ln

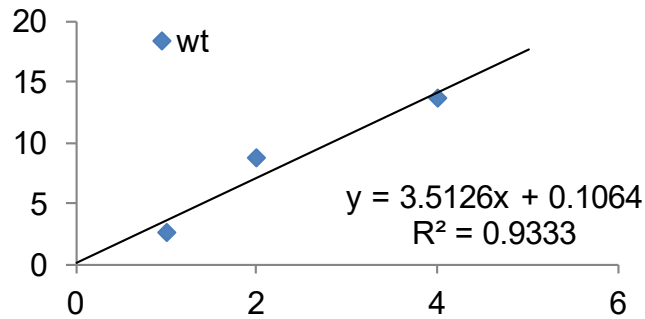


	IP fold	values	linear fit	divided	mean	% wt
wt	1	2,54	0,69	0,69		
	2	8,76	2,46	1,23		
	4	13,61	3,85	0,96	0,96	100,00

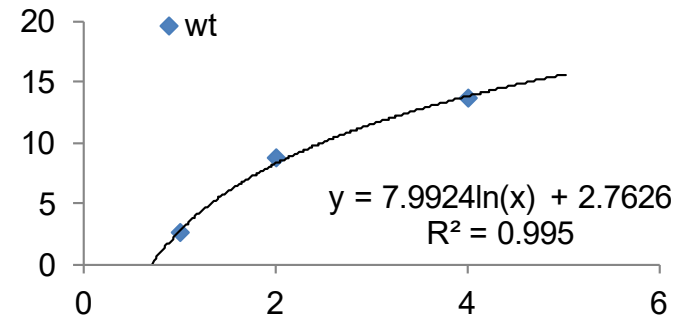
	IP fold	values	ln fit	divided	mean	% wt
wt	1	2,54	0,97	0,97		
	2	8,76	2,12	1,06		
	4	13,61	3,89	0,97	1,00	100,00



linear

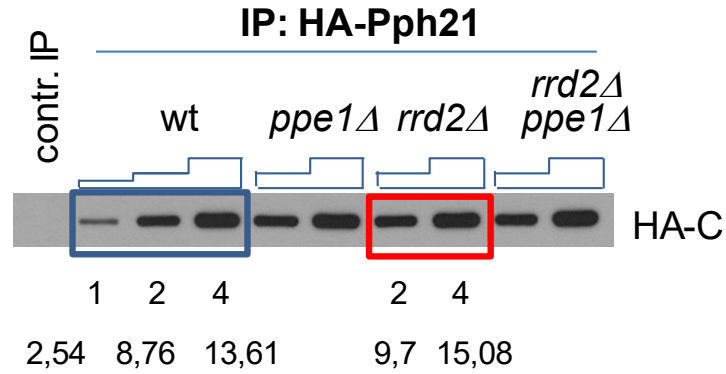


ln

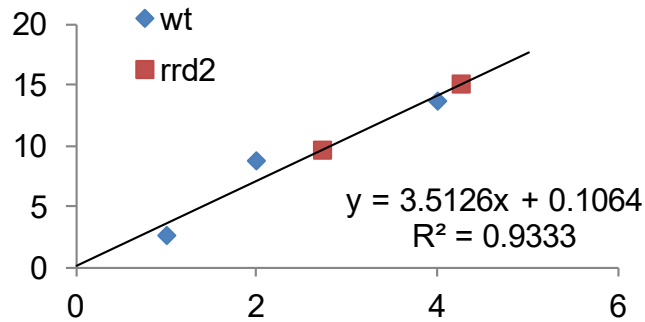


	IP fold	values	linear fit	divided	mean	% wt
wt	1	2,54	0,69	0,69		
	2	8,76	2,46	1,23		
	4	13,61	3,85	0,96	0,96	100,00

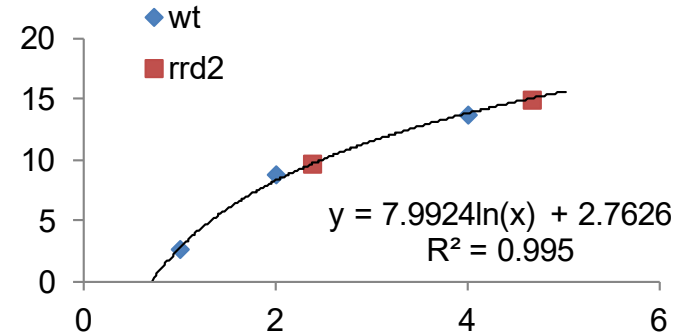
	IP fold	values	ln fit	divided	mean	% wt
wt	1	2,54	0,97	0,97		
	2	8,76	2,12	1,06		
	4	13,61	3,89	0,97	1,00	100,00



linear



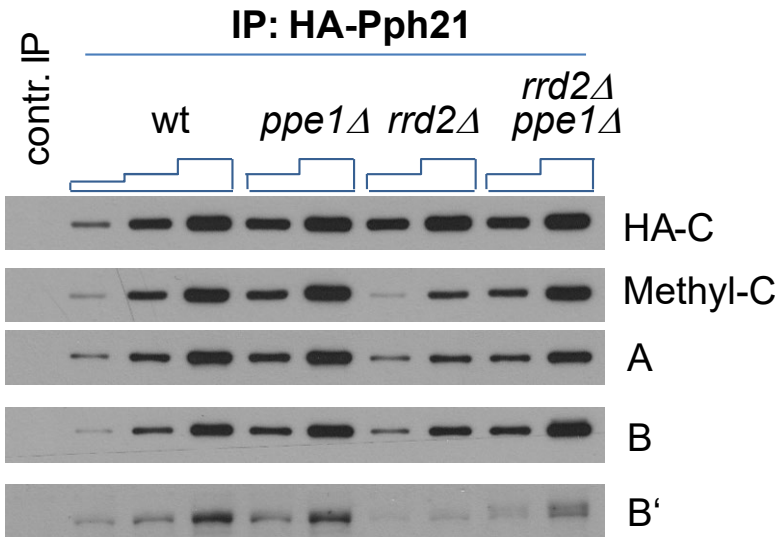
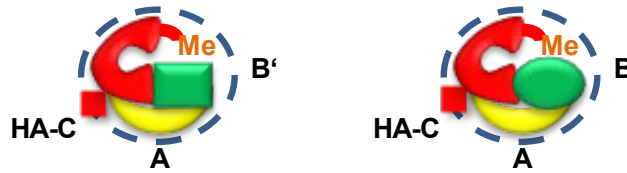
ln



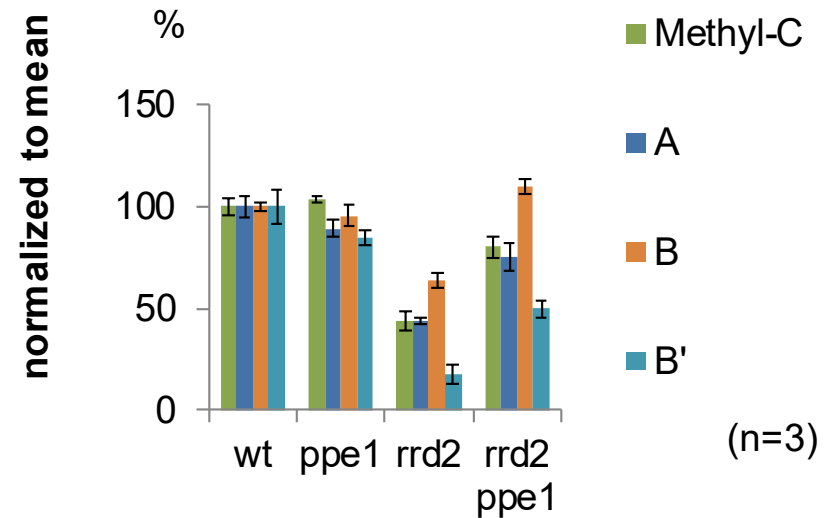
	IP fold	values	linear fit	divided	mean	% wt
wt	1	2,54	0,69	0,69		
	2	8,76	2,46	1,23		
	4	13,61	3,85	0,96	0,96	100,00
rrd2	2	9,70	2,73	1,37		
	4	15,08	4,26	1,07	1,22	126,46

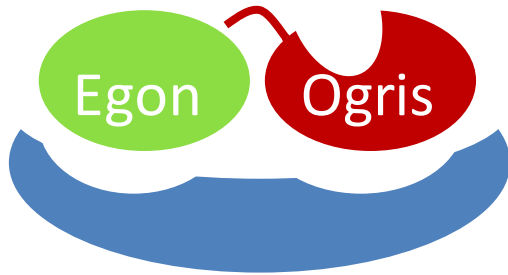
	IP fold	values	ln fit	divided	mean	% wt
wt	1	2,54	0,97	0,97		
	2	8,76	2,12	1,06		
	4	13,61	3,89	0,97	1,00	100,00
rrd2	2	9,70	2,38	1,19		
	4	15,08	4,67	1,17	1,18	117,83

IP of the PP2A complex



western blot quantification

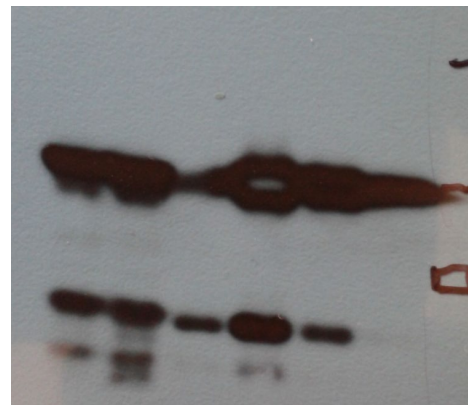




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western blot by Jiri Veis 2014