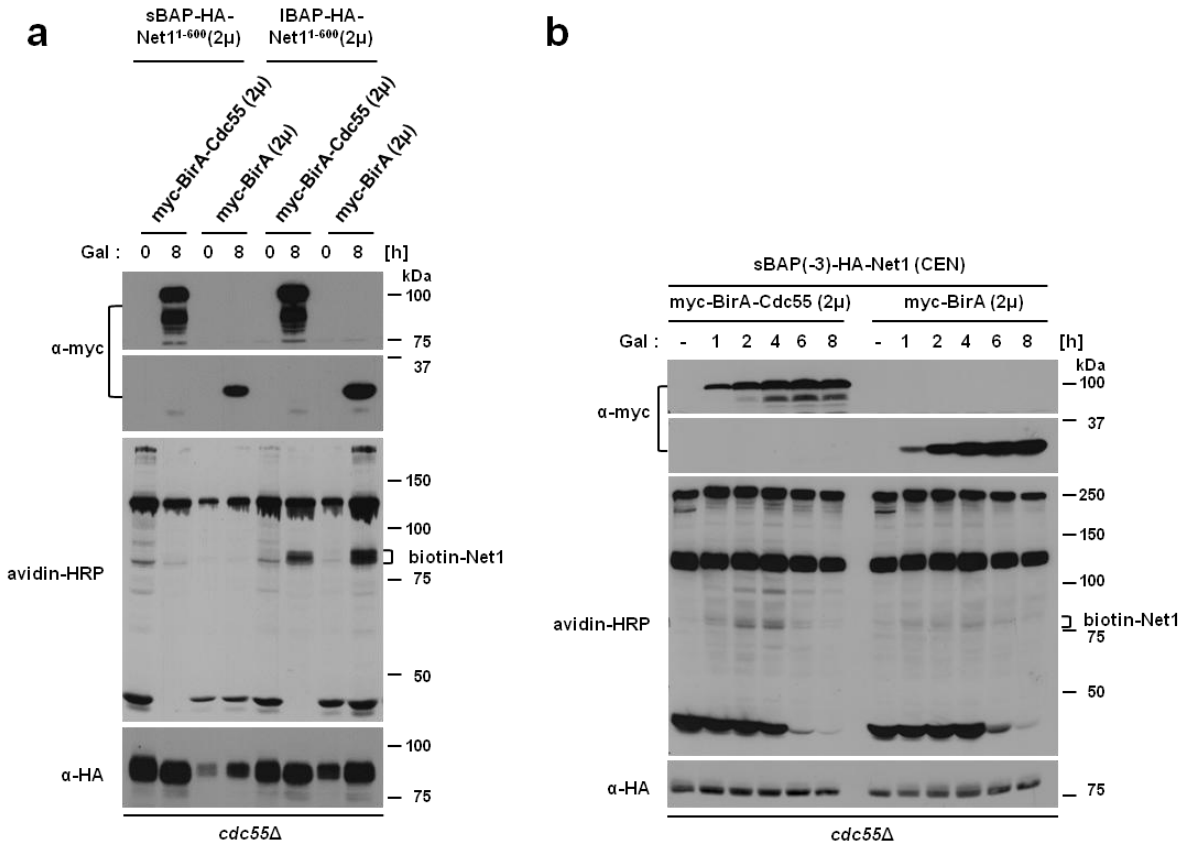
Supplementary Information for Zuzuarregui et al. "M-TRACK: an approach for detecting short-lived protein-protein interactions *in vivo*"

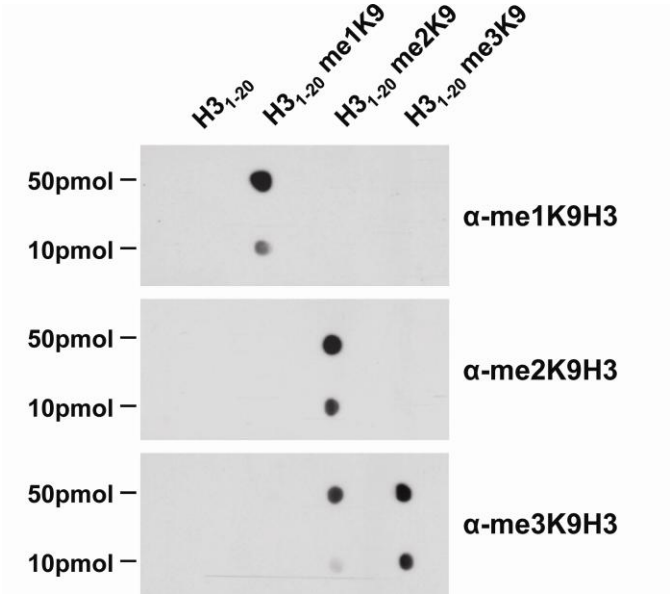
Supplementary Figures

Supplementary Figure 1: Biotin ligase assay of the interaction between PP2A-Cdc55 and Net1



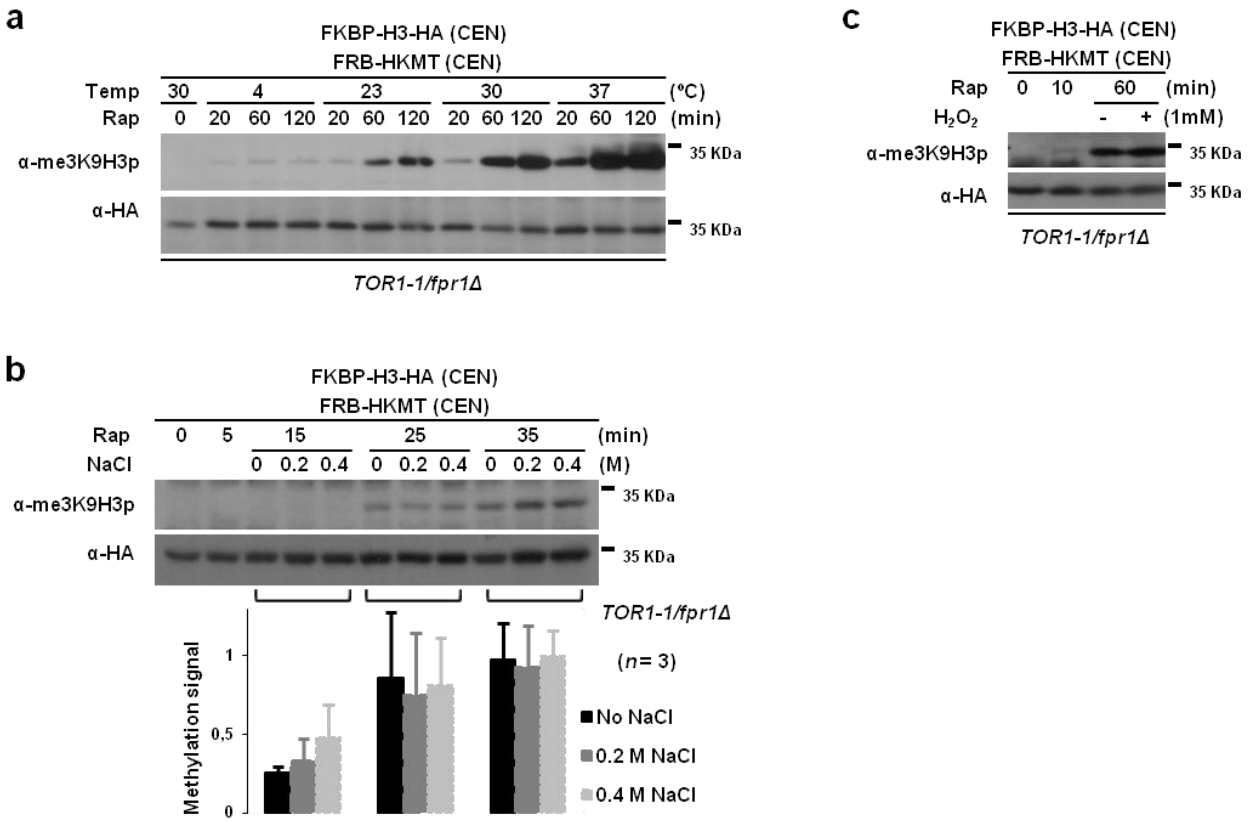
(**a**) At the indicated time of galactose induction, TCA lysates were prepared from a *cdc55*Δ strain expressing inducibly the bait myc-BirA-Cdc55 (2µ vector, PTK1) or myc-BirA (2µ vector, PTK2) and constitutively the prey sBAP-HA-Net1$^{1-600}$ (2µ vector; sBAP: GLNDIFEAQ**K**IEWHE, PTK3) (TKY106/TKY107) or lBAP-HA-Net1$^{1-600}$ (2µ vector; lBAP: MASSLRQILDSQ**K**MEWRSNAGGS, PTK4) (TKY108/TKY109). (**b**) At the indicated time of galactose induction, TCA lysates were prepared from a *cdc55*Δ strain expressing inducibly the bait myc-BirA-Cdc55 (2µ vector, PTK1) (YIF101) or myc-BirA (2µ vector, PTK2) (YIF 102), both strains constitutively expressing the prey sBAP(-3)-HA-Net1$^{1-600}$ (CEN vector; sBAP(-3): GLNDIFEAQ**K**IEGEF, PIF1). (**a,b**) Aliquots of lysates were analyzed by SDS-PAGE on separate gels, immunoblotted and incubated individually with avidin-HRP (1:5,000, Abcam) and specific antibodies against the HA- and the myc-tag.

Supplementary Figure 2: Specificity of histone H3-K9 monoclonal antibodies



Dot blot assay with the indicated amounts of peptides corresponding to amino acids 1-20 of histone H3 and bearing either unmodified Lys9 or mono-, di-, or tri-methylated Lys9. Peptides were detected with monoclonal antibodies raised against the respective Lys9 modifications.

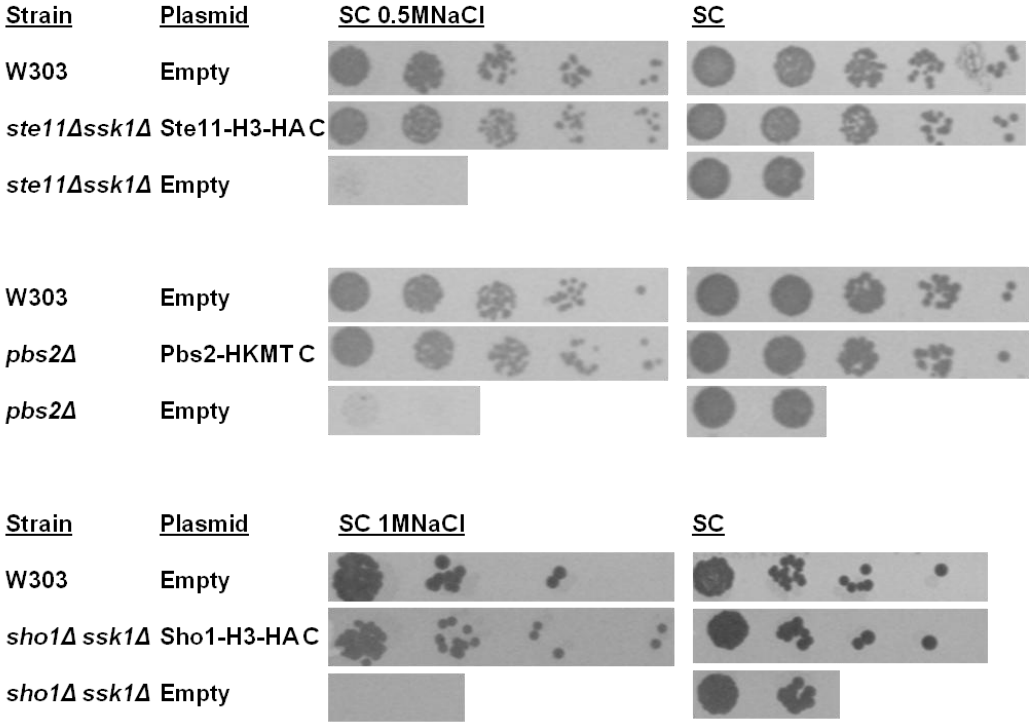
Supplementary Figure 3: Influence of various stress conditions on the assay system



FKBP-H3-HA (PC192) and FRB-HKMT (PC42) were coexpressed in a rapamycin resistance strain (*TOR1-1/fpr1*Δ, YC70). Dimerization of FKBP and FRB was induced with rapamycin (Rap). Protein samples were separated by 12 % SDS-PAGE, transferred to Nitrocelulose membranes (Amersham Pharmacia), blocked (5 % NFDM in PBS-T 0.1 %) and incubated with polyclonal trimethyl antibody (1:3,000 in 3 % BSA, 2 h at RT). Membranes were washed in PBS-T 0.1 %, incubated with the secondary antibody (1:10,000 α-rabbit-HRP (GE Healthcare) in PBS-T 0.1 %) washed again and analyzed by ECL. The membranes were subsequently stripped (10 min in 2 M $MgCl_2$, 0.1 M acetic acid at RT) and reprobed with HA antibody. (**a**) Testing for temperature sensitivity. Exponentially growing cultures at 30 °C were treated with rapamycin and immediately split into four parts that were grown at the indicated temperature. Samples were taken at the indicated time points and analyzed as described above. (**b**) Testing for salt sensitivity. After 5 minutes of rapamycin treatment samples were split and treated with water or salt at a final concentration of 0.2 and 0.4 M NaCl. Samples were taken at the indicated time points and analyzed as described above. This experiment was performed in triplicate and methylation and HA signal levels were quantified as described in the main methods section. 0 is the basal signal without induction,

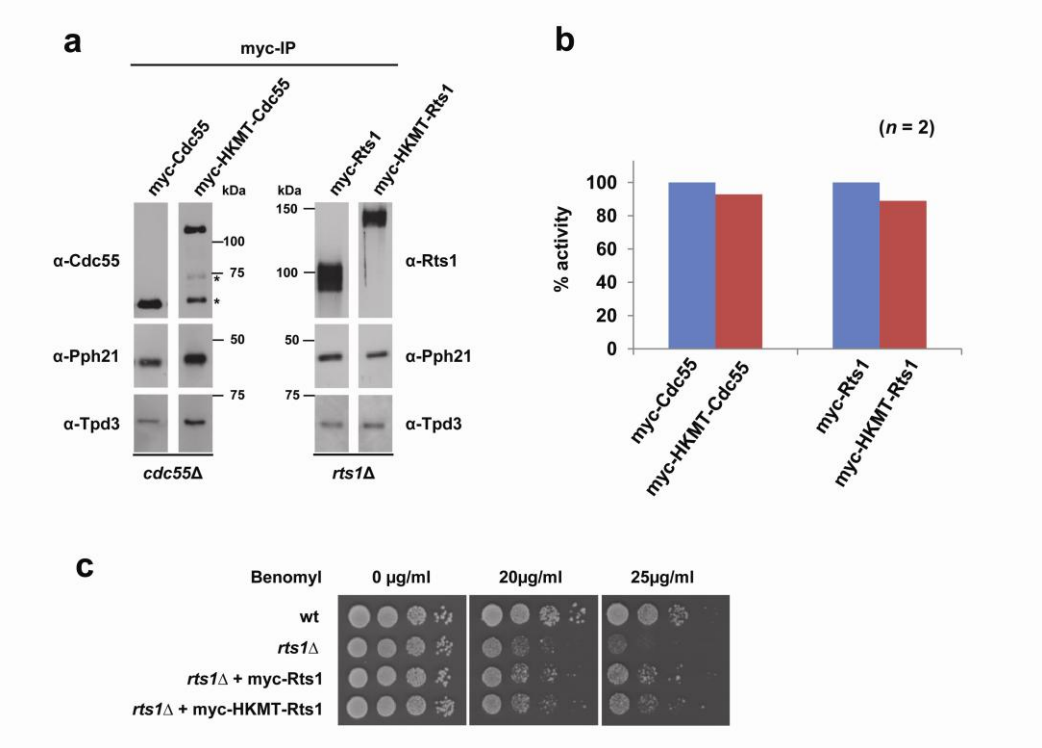
and 1 is the maximum signal measured in this experiment. One representative western blot is shown. (**c**) Testing for oxidative conditions. After 10 minutes of rapamycin treatment samples were split and one half was treated with 1 mM H_2O_2 for 50 minutes. Samples were taken at the indicated time points and analyzed as described above.

Supplementary Figure 4: Functionality of the tagged Hog1 pathway proteins *in vivo*



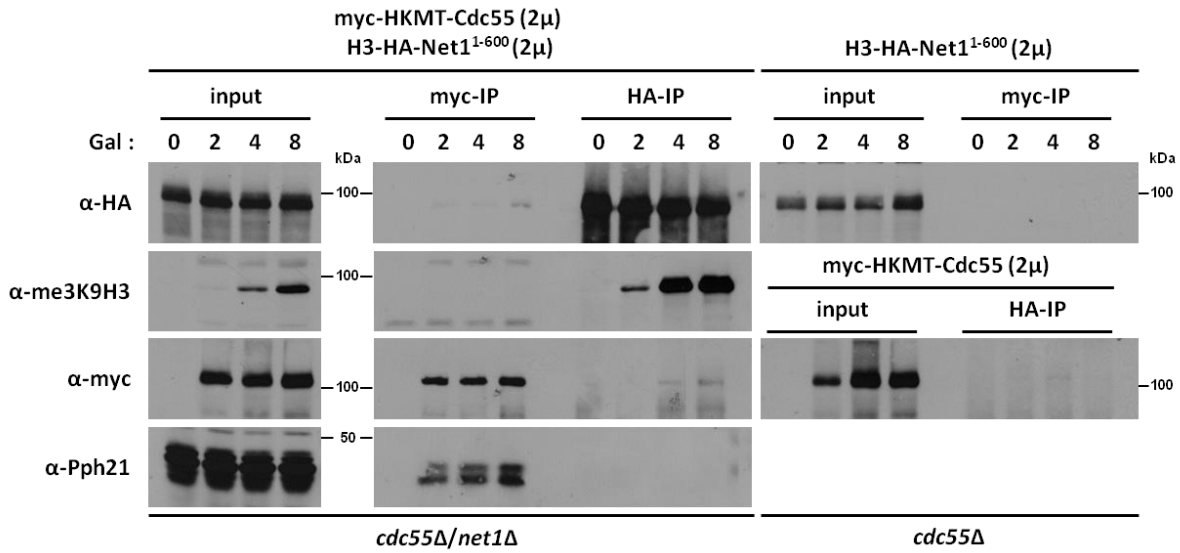
Ste11-H3-HA (CEN vector, PA119), Pbs2-HKMT (CEN vector, PC7), Hog1-HKMT (CEN vector, PC8) and Sho1-H3-HA (CEN vector, PI225) were transformed into *ste11*Δ/*ssk1*Δ (YC192), *pbs2*Δ (YVR10), *hog1*Δ (YPA185) or *sho1*Δ/*ssk1*Δ (YID155) strains, and salt sensitivity to 0.5 M NaCl (Ste11, Pbs2 and Hog1) or 1 M NaCl (Sho1) was tested by spotting different dilutions of exponentially growing cultures. As a positive control the wild-type strain was transformed with an empty plasmid and spotted onto the same plate. As a negative control the mutant strains were transformed with empty plasmids and also spotted onto the same plate.

Supplementary Figure 5: Functionality of the tagged PP2A proteins *in vitro* and *in vivo*



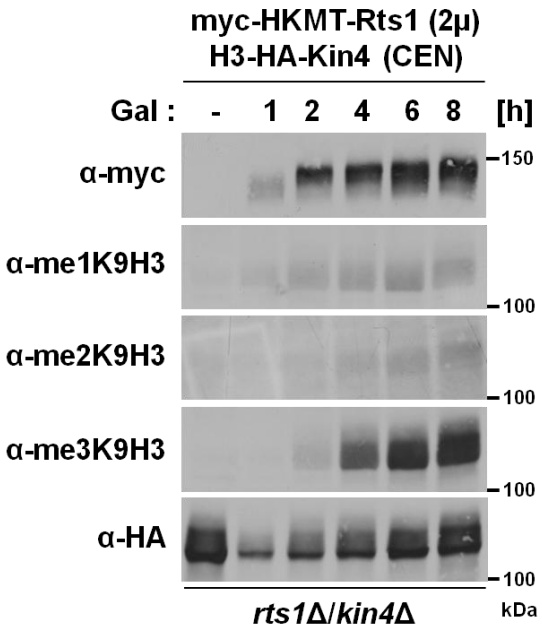
(**a**) Testing incorporation of the bait fusion-proteins into PP2A-holoenzymes. Anti-myc-immunoprecipitates from lysates of *cdc55*Δ and *rts1*Δ cells expressing either myc-Cdc55 (CEN vector, PIM101/YIM101), myc-Rts1 (CEN vector, PIM108/YIM103), myc-HKMT-Cdc55 (2µ vector, PIM102/YIM102) or myc-HKMT-Rts1 (2µ vector, PIM109/YIM104) were separated by SDS-PAGE and analyzed by immunoblotting with specific antibodies against Rts1, Cdc55, Pph21 and Tpd3. The anti-Pph21 blot was reprobed with anti-Tpd3, while separate blots were used for the detection of Cdc55 and Rts1. Asterisks indicate degradation products. (**b**) *In vitro* phosphatase assay towards ^{32}P-labeled phosphorylase a with immunoprecipitated PP2A complexes (obtained in the experiments shown in Supplementary Fig. 3a) (*n* = 2, mean of specific catalytic activity percentage, 100 % corresponds to the specific activity obtained by complexes containing myc-Cdc55 or myc-Rts1, respectively). (**c**) Testing *in vivo* functionality of the myc-HKMT-Rts1 fusion protein. Logarithmically growing cultures of wt (BY4741), *rts1*Δ (Y01790) and *rts1*Δ expressing myc-Rts1 (CEN vector, PIM108/YIM103) or myc-HKMT-Rts1 (2µ vector, PIM109/YIM104) were 10-fold serially diluted in YPD liquid medium, spotted on YPD-plates containing the indicated amounts of benomyl and incubated for 2-3 days at 30 °C.

Supplementary Figure 6: Immunoprecipitation assays with H3-HA-Net1$^{1-600}$ and myc-HKMT-Cdc55



Anti-HA- and anti-myc-immunoprecipitates from lysates of *cdc55*Δ/*net1*Δ cells expressing H3-HA-Net1$^{1-600}$ (2µ vector, PIM105) and myc-HKMT-Cdc55 (2µ vector, PIM103) (TKY100) were analyzed by SDS-PAGE and immunoblotted with the indicated antibodies. Anti-myc-immunoprecipitates of *cdc55*Δ cells expressing only H3-HA-Net1$^{1-600}$ (2µ vector, PIM105) (TKY105) and anti-HA-immunoprecipitates of *cdc55*Δ cells expressing only myc-HKMT-Cdc55 (2µ vector, PIM103) (TKY104) served as negative controls. Input correlates to 1/20 of the lysate amounts used for the respective IP. The anti-myc incubated blot was reprobed with anti-me3K9H3 to test for the M-TRACKing of the Cdc55-Net1 interaction; the anti-HA incubated blot was reprobed with anti-Pph21 to test for PP2A holoenzyme assembly. Panels originate from the same exposure of the same blotting membrane incubated with the indicated antibody.

Supplementary Figure 7: M-TRACKing of the interaction between PP2A-Rts1 and Kin4



At the indicated time-points of galactose induction, TCA lysates were prepared from a *rts1Δ/kin4Δ* strain expressing inducibly the bait myc-HKMT-Rts1 (2µ vector, PIM107) and constitutively the prey H3-HA-Kin4 (CEN vector, PBB1) (YBB14). Lysates were analyzed by 7.5 % SDS-PAGE, immunoblotted onto separate membranes and incubated individually with specific antibodies against mono-, di-, tri-methylated K9H3, the HA- and the myc-tag.

Supplementary Tables

Supplementary Table 1: *Saccharomyces cerevisiae* strains used in this study

Name	Relevant genotype	Source or reference
W303	Mat a*, ade2, trp1, can1, leu2, his3, ura3*	Rodney Rothstein
YC8	W303 *ssk2::KanMx ssk22::KanMx*	S. Hohmann
YC70	W303 *TOR1-1 fpr1::NatMx*	This study
YC192	W303 *ste11::ade ssk1::his*	This study
YC232	W303 *sho1::his pbs2::kan TOR1-1 fpr1::NatMx*	This study
YID140	W303 *ste11::ade pbs2::his*	This study
YID155	W303 *sho1::trp ssk1::leu*	This study
YA151	W303 *sho1::trp pbs2::his ssk2::KanMx ssk22::KanMx*	This Study
YVR10	W303 *pbs2::his*	[1]
YPA185	W303 *hog1::kan*	Paula Alepuz
BY4741	Mat a; *his3*Δ1; *leu2*Δ; *met15*Δ0; *ura3*Δ1	Euroscarf
YIM100	BY4741 *YGL190c::HIS3MX6*; pYES2-myc-HKMT-Cdc55-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
YIM101	BY4741 *YGL190c::HIS3MX6*; pYX142-myc-Cdc55-URA	This study
YIM102	BY4741 *YGL190c::HIS3MX6*; pYX242-myc-HKMT-Cdc55-URA	This study
TKY100	BY4741 *YGL190c::HIS3MX6*; *YJL076W::natNT2*; pYX213-myc-HKMT-Cdc55-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
TKY101	BY4741 *YGL190c::HIS3MX6*; *YJL076W::natNT2*; pYES2-myc-HKMT-Cdc55-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
TKY102	BY4741 *YGL190c::HIS3MX6*; *YJL076W::natNT2*; pYES2-myc-HKMT-Cdc55-URA; pYX242-4H3-2HA-Net1(1-600)(S166A,T212A,S252A)-LEU	This study
TKY103	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-HKMT-Cdc55-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
TKY104	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-HKMT-Cdc55-URA	This study
TKY105	BY4741 *YGL190c::HIS3MX6*; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
YIM103	BY4741 *YOR014W::kanMX4*; pYX142-myc-Rts1-URA	This study
YIM104	BY4741 *YOR014W::kanMX4*; pYX242-myc-HKMT-Rts1-URA	This study
YIM105	BY4741 *YGL190c::HIS3MX6*; pYES2-myc-HKMT-Cdc55-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
YBB14	BY4741 *YOR014W::kanMX4*; YOR233W::natNT2; pYX213-myc-HKMT-Rts1-URA; pYX142-4H3-2HA-Kin4-LEU	This study
YBB19	BY4741 *YOR014W::kanMX4*; pYX213-myc-HKMT-Rts1-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study

YBB26	BY4741 pYES2-myc-HKMT-GL-URA; pYX242-3H3-2HA-Net1(1-600)-LEU	This study
YPP1	BY4741 *YGL190c::HIS3MX6*	[2]
Y01790 (Acc.no.)	BY4741 *YOR014W::kanMX4*	Euroscarf
TKY106	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-BirA-GL-Cdc55, pYX242-SBAP-2HA-GL-Net1(1-600)	This study
TKY107	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-BirA-GL, pYX242-SBAP-2HA-GL-Net1(1-600)	This study
TKY108	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-BirA-GL-Cdc55, pYX242-lBAP-2HA-GL-Net1(1-600)	This study
TKY109	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-BirA-GL, pYX242-lBAP-2HA-GL-Net1(1-600)	This study
YIF101	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-BirA-GL-Cdc55, pYX142-sBAP(-3)-HA-GL-Net1(1-600)	This study
YIF102	BY4741 *YGL190c::HIS3MX6*; pYX213-myc-BirA-GL, pYX142-sBAP(-3)-HA-GL-Net1(1-600)	This study

Supplementary Table 2: Plasmids used in this study

Plasmid	Description	Source or reference
PC1	Pbs2-FKBP-YCp22	This study
PC7	Pbs2-HKMT-YCp111	This study
PC8	Hog1-HKMT-YCp111	This study
PC14	Ste20-HKMT-YCp111	This study
PC18	Ste50-HKMT-YCp111	This study
PC30	Rga1-HKMT-YCp111	This study
PC42	*ADH1*p-FRB-HKMT-YCp111	This study
PC54	Sho1-HKMT-YCp111	This study
PC157	Bem3-HKMT-YCp111	This study
PC160	*ADH1*p-Fus1-HKMT-YCp111	This study
PC165	HKMT-Cdc42-YCp111	This study
PC181	4H3-3HA-Cdc42-YEp195	This study
PC192	*ADH1*p-4H3-3HA-FKBP-YCp22	This study
PI225	Sho1-4H3-3HA-YCp33	This study
PI228	Sho1-4H3-3HA-YEp195	This study
PI389	Sho1Y8A-4H3-3HA-YCp33	This study
PI395	Sho1Y54M-4H3-3HA-YCp33	This study
PA119	Ste11-4H3-3HA-YCp33	This study
PIM100	pYES2-myc-HKMT-GL-Cdc55	This study
PIM101	pYX142-myc-GL-Cdc55	This study
PIM102	pYX242-myc-HKMT-GL-Cdc55	This study
PIM103	pYX213-myc-HKMT-GL-Cdc55	This study
PIM104	pYES2-myc-HKMT-GL	This study
PIM105	pYX242-3H3-2HA-GL-Net1(1-600)	This study
PIM106	pYX242-4H3-2HA-GL-Net1(1-600)(S166A,T212A,S252A)	This study
PIM107	pYX213-myc-HKMT-GL-Rts1	This study
PIM108	pYX142-myc-GL-Rts1	This study
PIM109	pYX242-myc-HKMT-GL-Rts1	This study
PBB1	pYX142-4H3-2HA-GL-Kin4	This study
PTK1	pYX213-myc-BirA-GL-Cdc55	This study
PTK2	pYX213-myc-BirA-GL	This study
PTK3	pYX242-sBAP-2HA-GL-Net1(1-600)	This study
PTK4	pYX242-lBAP-2HA-GL-Net1(1-600)	This study
PIF1	pYX142-sBAP(-3)-HA-GL-Net1(1-600)	This study

Supplementary Table 3: Antibodies used in this study

Antibody	Description	Dilution for WB	Source
α-me3K9H3p[(A)]	Rabbit polyclonal, n°2236	1:3000	T. Jenuwein
α-me3K9H3	Mouse monoclonal, clone 6F12-H4	1:100 to 1:500[(E)]	Millipore
α-me2K9H3	Mouse monoclonal, clone 5E5-G5	1:20 to 1:100	This study
α-me1K9H3	Mouse monoclonal, clone 7E7-H12	1:50 to 1:100	This study
α-rabbit-HRP[(B)]		1:10,000	GE Healthcare
α-rabbit-HRP[(C)]		1:10,000	Jackson ImmunoResearch
α-mouse-HRP		1:10,000	Jackson ImmunoResearch
α-HA	Mouse monoclonal, clone 16B12	1:20,000	Covance research products
α-HA[(D)]	Mouse monoclonal, clone 12CA5	1:5,000	[3]
α-P-Hog	Rabbit polyclonal, α-P-p38 MAPK T180/Y182	1:4,000	Cell Signaling
α-myc	Mouse monoclonal, clone 4A6	1:1,000[(E)]	Millipore
α-Pph21	Rabbit polyclonal	1:10,000	[3]
α-Tpd3	Mouse monoclonal, clone 5G2	1:200	[2]
α-Cdc55	Mouse monoclonal, clone 9D3H6	1:300	This study
α-Rts1	Rabbit polyclonal	1:10,000	This study

(A) Used for supplementary figure 3

(B) Used for HOG studies

(C) Used for PP2A studies

(D) Used for HA-Kin4 detection

(E) Dilution applies to supernatant produced in the laboratory

Supplementary Notes

Supplementary Note 1: Biotin ligase assay

A protein proximity assay based on biotin ligase (BirA) and a BirA-acceptor peptide (BAP) has been described recently in mammalian cells [4, 5]. No experimental evidence, however, has been provided for the ability of the biotin system to detect short-lived interactions. Thus, we tested the biotin ligase system for the ability to detect the enzyme-substrate interaction between PP2A-Cdc55 and its substrate Net1 (Supplementary Fig. 1a,b). We tagged Cdc55 N-terminally with the BirA-ligase and Net1 with 3 different versions of the BirA-acceptor site, short BAP (sBAP), long BAP (lBAP) (Supplementary Fig. 1a) and a mutant short BAP (sBAP(-3)) (Supplementary Fig. 1b). The latter had been generated by Fernández-Suárez et al. because of the high background detection these authors obtained with the short BAP substrate, which was due to the high affinity between BirA and sBAP.

In agreement with their data on the high background and unspecific detection we found that BirA on its own biotinylated the prey lBAP-Net1 to similar levels as the BirA-Cdc55 fusion protein. Moreover, the sBAP- and sBAP(-3)-tagged Net1 were hardly detectable with either BirA or BirA-Cdc55 despite the considerable expression of the bait and prey fusion proteins (with the exception of the very low sBAP-tagged Net1 levels in the BirA ligase only expressing strain; Supplementary Fig. 1a). Based on these and the published data, we think that the BirA ligase system - in contrast to M-TRACK - may not be suitable for detection of short-lived and/or dynamic protein interactions.

Supplementary Note 2: Sequences

Sequence of the HKMT SET domain mutant H320R (amino acids 82-412) used for the methylation assay (the mutated amino acid is highlighted in bold):

CVRILKQFHKDLERELLRRHHRSKTPRHLDPSLANYLVQKAKQRRALRRWEQELNAKRSHLG
RITVENEVDLDGPPRAFVYINEYRVGEGITLNQVAVGCECQDCLWAPTGGCCPGASLHKFAY
NDQGQVRLRAGLPIYECNSRCRCGYDCPNRVVQKGIRYDLCIFRTDDGRGWGVRTLEKIRKN
SFVMEYVGEIITSEEAERRGQIYDRQGATYLFDLDYVEDVYTVDAAYYGNIS**R**FVNHSCDPN
LQVYNVFIDNLDERLPRIAFFATRTIRAGEELTFDYNMQVDPVDMESTRMDSNFGLAGLPGS
PKKRVRIECKCGTESCRKYLF

Sequence of the 4xH3 3xHA tag (the first of the four H3-repeats is highlighted in bold):

gggatcccgaattcg**gctcgtactaagcagaccgctcgcaagtccaccggcggcaaggcc**
G I P N S **A R T K Q T A R K S T G G K A**

ccgcgcaagcagctggcccagatcccgaattcggctcgtactaagcagaccgctcgcaag
P R K Q L A Q I P N S A R T K Q T A R K

tccaccggcggcaaggccccgcgcaagcagctggcccagatcccgaattcggctcgtact
S T G G K A P R K Q L A Q I P N S A R T

aagcagaccgctcgcaagtccaccggcggcaaggccccgcgcaagcagctggcccagatc
K Q T A R K S T G G K A P R K Q L A Q I

ccgaattcggctcgtactaagcagaccgctcgcaagtccaccggcggcaaggccccgcgc
P N S A R T K Q T A R K S T G G K A P R

aagcagctggcccagatctgcggccgcatcttttacccatacgatgttcctgactatgcg
K Q L A Q I C G R I F Y P Y D V P D Y A

ggctatccctatgacgtcccggactatgcaggatcctatccatatgacgttccagattac
G Y P Y D V P D Y A G S Y P Y D V P D Y

gctgctcagtgcggccgc
A A Q C G R

Supplementary References

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