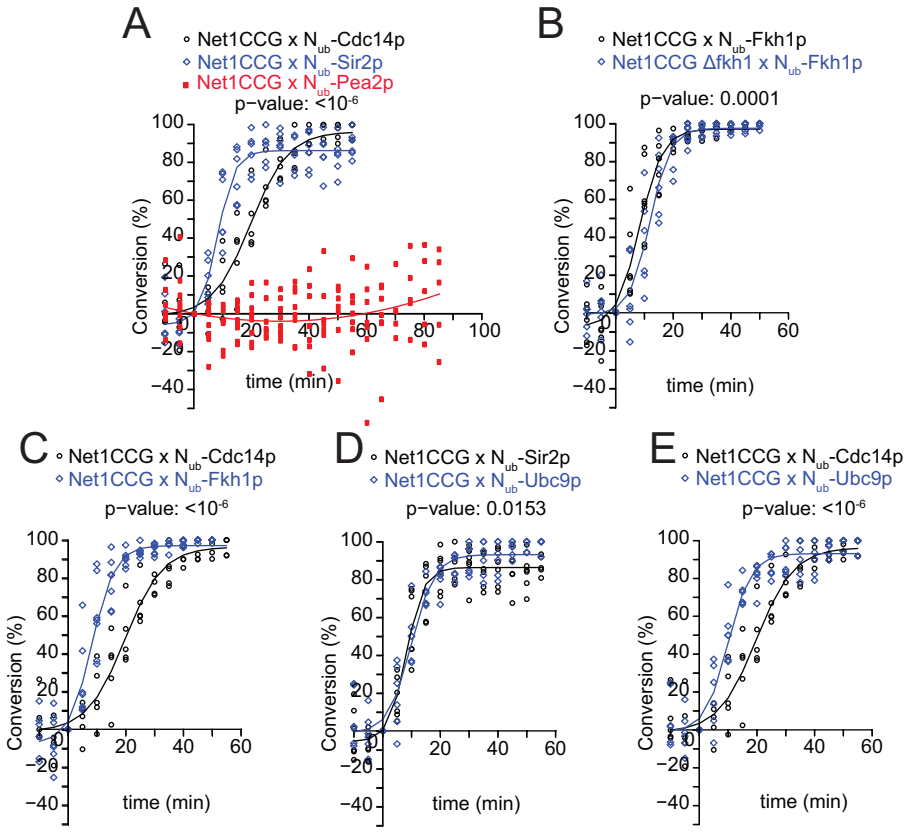
Supplementary Information for

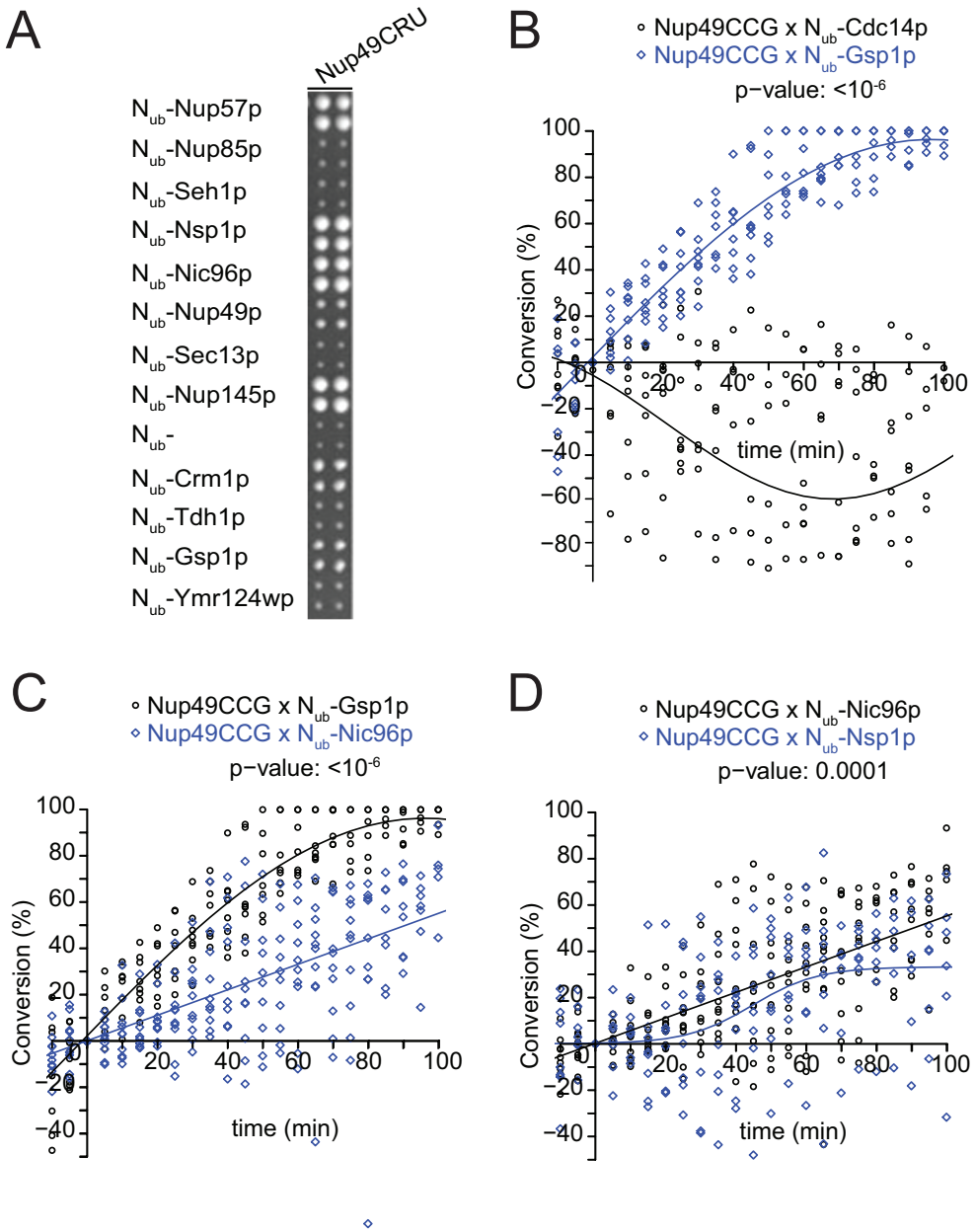
A fluorescent reporter for mapping cellular protein-protein interactions in time and space

Table of Content:

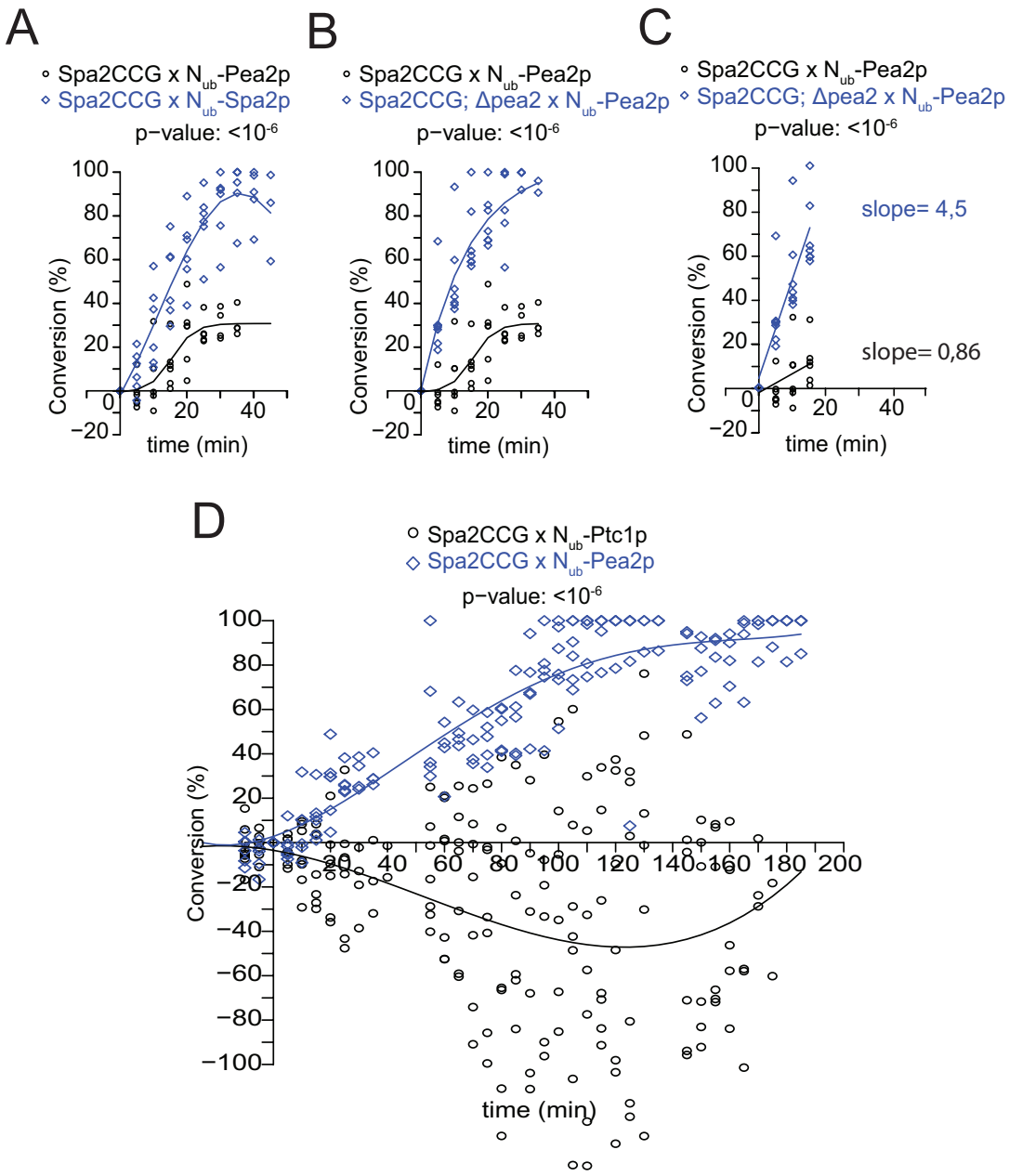
The Supplementary Information contains four figures, ten movies, two tables, and one reference.



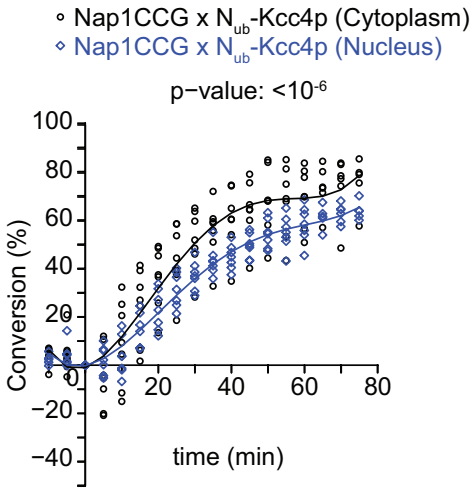
Supplementary Figure 1
Interactions of Net1p. Related to Figure 2.
Shown are the pairwise comparisons of the time dependent conversion of Net1CCG by different N_{ub}-fusion proteins. Lines show best fits for each interaction profile selected via the Akaike Information Criterion after fitting multiple models. P-values are calculated from the F-test. Net1CCG was expressed in diploid cells derived from either wildtype a-cells (a, c, d, e) or a-cells lacking *FKH1* (b, blue symbol and line).



Supplementary Figure 2
Split-Ubiquitin analysis of interations at the nuclear pore. Related to Figure 4. (a) Split-Ubiquitin growth assay of diploid cells expressing Nup49CRU together with N_{ub}-fusions of other members of the nuclear pore, of members of the nucleo-cytoplamic traffic, and with N_{ub}-fusions of unrelated proteins. Shown are the quadruplets of four independent matings each on selective medium containing 5-FOA. (b-d) Pairwise comparisons of the time dependent conversion of Nup49CCG by different N_{ub}-fusion proteins. Lines show best fits for each interaction profile selected via the Akaike Information Criterion after fitting multiple models. P-values are calculated from the F-test.



Supplementary Figure 3
Intactions at the polar cortical domain. Related to Figure 5.
Shown are the pairwise comparisons of the time dependent conversion of Spa2CCG by N_{ub}-Pea2p (a, b) and N_{ub}-Ptc1p (d). Lines show best fits for each interaction profile selected via the Akaike Information Criterion after fitting multiple models. P-values are calculated from the F-test. Spa2CCG was expressed in diploid cells derived from either wildtype a-cells (a, d,) or a-cells lacking *PEA2* (b, blue symbol and line). (c) Linear regression lines and slopes for the first 15 min of Spa2CCG conversion induced by N_{ub}-Pea2p in the presence (black symbol and line) and absence of native *PEA2* (blue symbol and line)



Supplementary Figure 4
Interactions of Nap1 in the nucleus and the cytoplasm. Related to Figure 7. Shown is the pairwise comparison of the time dependent conversion of Nap1CCG by N_{ub}-Kcc4p in the cytoplasm (black symbol and line) and the nucleus (blue symbol and line) of the mated diploids. Lines show best fits for each interaction profile selected via the Akaike Information Criterion after fitting multiple models. P-values are calculated from the F-test.

Description of Supplementary Movies

Supplementary Movie 1

Time lapse analysis of the mating of two yeast cells expressing Net1CCG and N_{ub}-Sir2p. Related to Figure 2. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point of highest fluorescence intensity at the beginning of cell fusion. Scale bar, 5 μm.

Supplementary Movie 2

Time lapse analysis of the mating of two yeast cells expressing Net1CCG and N_{ub}-Cdc14p respectively. Related to Figure 3. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point of highest fluorescence intensity at the beginning of cell fusion. Scale bar, 5 μm.

Supplementary Movie 3

Time lapse analysis of the mating of two yeast cells expressing Nup49CCG and N_{ub}-Gsp1p. Related to Figure 4. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point shortly before fusion of the nuclei. Scale bar, 5 μm. N_{ub}-Gsp1p expression was induced by 100 μM copper.

Supplementary Movie 4

Time lapse analysis of the mating of two yeast cells expressing Nup49CCG and N_{ub}-Nsp1p respectively. Related to Figure 4. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point shortly before fusion of the nuclei. Scale bar, 5 μm. N_{ub}- Nsp1p expression was induced by 100 μM copper

Supplementary Movie 5

Time lapse analysis of the mating of two yeast cells expressing Spa2CCG and N_{ub}-Pea2p. Related to Figure 5. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point of highest fluorescence intensity at the beginning of cell fusion. Scale bar, 5 μm.

Supplementary Movie 6

Time lapse analysis of the mating of two yeast cells expressing Spa2CCG and N_{ub}-Spa2p respectively. Related to Figure 5. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point of highest fluorescence intensity at the beginning of cell fusion. Scale bar, 5 μm.

Supplementary Movie 7

Time lapse analysis of the mating of two yeast cells expressing Spa2CCG and N_{ub}-Pea2p. Related to Figure 5. The Spa2CCG expressing strain lacks *PEA2*. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point of highest fluorescence intensity at the beginning of cell fusion. Scale bar, 5 μm.

Supplementary Movie 8

Time lapse analysis of the mating of two yeast cells expressing Spa2CCG and N_{ub}-Hof1p. Related to Figure 5. Time 0 indicates the time point of highest fluorescence intensity at the beginning of cell fusion. Scale bar, 5 μm.

Supplementary Movie 9

Time lapse analysis of the mating of two yeast cells expressing Nap1CCG and N_{ub}-Kcc4p. Related to Figure 6. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point shortly before cell fusion. Scale bar, 5 μm. N_{ub}- Kcc4p expression was induced by 100 μM copper. Images were deconvoluted before preparation of the movie.

Supplementary Movie 10

Time lapse analysis of the mating of two yeast cells expressing Stu2CCG and N_{ub}-Kar9p. Related to Figure 8. Shown is the merge of Cherry- and GFP-fluorescence. Time 0 indicates the time point when the two SPBs align to each other. Scale bar, 5 μm.

Supplementary Table 1

List of constructs used and created in this study

Name	Description	Source
NET1-C_{Ub}-RURA3 pRS303	*lacZ Amp^r HIS3*	This study
NUP49-C_{Ub}-RURA3 pRS303	*lacZ Amp^r HIS3*	This study
SPA2C_{Ub}-RURA3 pRS303	*lacZ Amp^r HIS3*	This study
STU2-C_{Ub}-RURA3 pRS303	*lacZ Amp^r HIS3*	This study
NET1-Cherry-C_{Ub}-RGFP pRS306	*lacZ Amp^r URA3*	This study
NUP49-Cherry-C_{Ub}-RGFP pRS306	*lacZ Amp^r URA3*	This study
SPA2-Cherry-C_{Ub}-RGFP pRS306	*lacZ Amp^r URA3*	This study
KEL1-Cherry-C_{Ub}-RGFP pRS306	*lacZ Amp^r URA3*	This study
NAP1-Cherry-C_{Ub}-RGFP pRS306	*lacZ Amp^r URA3*	This study
STU2-Cherry-C_{Ub}-RGFP pRS306	*lacZ Amp^r URA3*	This study

Supplementary Table 2

List of yeast strains used and created in this study

Strain	Name	Genotype	Source
JD53	JD53	*Matα ura3-52 leu2-3,112 his3-Δ200 lys2-801 trp1-Δ63*	Dohmen et al., 1995
JD47-13c	JD47-13c	*Mata ura3-52 leu2-3,112 his3-Δ200 lys2-801 trp1-Δ63*	Dohmen et al., 1995
JD51	JD51	*Mata/α ura3-52/ura3-53 leu2-3,112/ leu2-3,112 his3-Δ200/ his3-Δ200 lys2-801/lys2-801 trp1-Δ63/trp1-Δ63*	Dohmen et al., 1995
YAD196	Net1CRU	JD47-13c, *NET1::NET1-C_{UB}-RURA3 pRS303*	This study
YAD552	Nup49CRU	JD47-13c, *NUP49::NUP49-C_{UB}-RURA3 pRS303*	This study
UNY97	Spa2CRU	JD47-13c, *SPA2::SPA2C_{UB}-RURA3 pRS303*	This study
STY225	Stu2CRU	JD47-13c, *STU2::STU2C_{UB}-RURA3 pRS303*	This study
YAD491	Net1CCG	JD47-13c, *NET1::NET1-CHERRY-C_{UB}-RGFP pRS306*	This study
YAD492	Nup49CCG	JD47-13c, *NUP49::NUP49-CHERRY-C_{UB}-RGFP pRS306*	This study
YAD424	Spa2CCG	JD47-13c, *SPA2::SPA2-CHERRY-C_{UB}-RGFP pRS306*	This study
STY332	Kel1CCG	JD47-13c, *KEL1::KEL1-CHERRY-C_{UB}-RGFP pRS306*	This study
MZY115	Nap1CCG	JD47-13c, *NAP1::NAP1-CHERRY-C_{UB}-RGFP pRS306*	This study
STY359	Stu2CCG	JD47-13c, *STU2::STU2-CHERRY-C_{UB}-RGFP pRS306*	This study
YAD662	Net1CCG Δfkh1	JD47-13c, *NET1::NET1-CHERRY-C_{UB}-RGFP pRS306, FKH1::hphNT1Δfkh1*	This study
YAD670	Spa2CCG Δpea2	JD47-13c, *SPA2::SPA2-CHERRY-C_{UB}-RGFP pRS306, PEA2::hphNT1Δpea2*	This study
YAD663	Spa2CCG Δhof1	JD47-13c, *SPA2::SPA2-CHERRY-C_{UB}-RGFP pRS306, HOF1::natNΔhof1*	This study
YAD671	Spa2CCG Δymr124w	JD47-13c, *SPA2::SPA2-CHERRY-C_{UB}-RGFP pRS306, YMR124w::hphNT1Δymr124w*	This study
Nub85	N_{ub}-Sir2	JD53, *P_{SIR2}::kanMX6 $P_{CUP1}N_{ub}$-HA*	This study
SHY159	N_{ub}-Ubc9	JD53, *P_{UBC9}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY31	N_{ub}-Cdc14	JD53, *P_{CDC14}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY70	N_{ub}-Fkh1	JD53, *P_{FKH1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY86	N_{ub}-Tdh1	JD53, *P_{TDH1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
Nub99	N_{ub}-Nup57	JD53, *P_{NUP57}::kanMX6 $P_{CUP1}N_{ub}$-HA*	This study
Nub103	N_{ub}-Nic96	JD53, *P_{NIC96}::kanMX6 $P_{CUP1}N_{ub}$-HA*	This study
Nub102	N_{ub}-Nsp1	JD53, *P_{NSP1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	This study
Nub107	N_{ub}-Gsp1	JD53, *P_{GSP1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	This study
SHY156	N_{ub}-Spa2	JD53, *P_{SPA2}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
Nub69	N_{ub}-Pea2	JD53, *P_{PEA2}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011

SHY178	N_{ub}-Ptc1	JD53, *P_{PTC1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY248	N_{ub}-Hof1	JD53, *P_{HOF1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
KLY122	N_{ub}-Hof1$_{98-609}$	JD53, *$HOF1_{1-100}$::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY106	N_{ub}-YMR124w	JD53, *$P_{YMR124w}$::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY216	N_{ub}-Kel1	JD53, *P_{KEL1}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY207	N_{ub}-Kcc4	JD53, *P_{KCC4}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
SHY198	N_{ub}-Kar9	JD53, *P_{KAR9}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
TAY8	N_{ub}-Spc72	JD53, *P_{SPC72}::kanMX6 $P_{CUP1}N_{ub}$-HA*	Hruby *et al.*, 2011
YAD305	N_{ub}-Spa2 Δpea2	JD53, *P_{SPA2}::kanMX6 $P_{CUP1}N_{ub}$-HA, PEA2::CmLEU2Δpea2*	This study
YAD688	P_{CUP1}GFP-Cdc14	JD53, *P_{CDC14}::kanMX6P_{CUP1}GFP*	This study

Supplementary References

Dohmen, R. J., Stappen, R., McGrath, J. P., Forrova, H., Kolarov, J., Goffeau, A., Varshavsky, A. (1995). An essential gene encoding a homolog of ubiquitin-activating enzyme. *J. Biol. Chem.* **270**: 18099-18109.