Table S1 - Overview of FCS data presented in this study (corresponding to Fig.1)

	Number of measurements	Particle concentration		Diffusion coefficient	
	N	nM	SD	µm^2/sec	SD
1myEGFP	53	1103	376	8,72	3,66
3myEGFP	30	1173	272	5,72	1,21
Abp1 3myEGFP	5	1206	403	2,57	0,47
Apl1 1myEGFP	24	33	19	1,67	0,46
Apl3 3myEGFP	33	47	19	1,28	0,63
Apm4 3myEGFP	12	32	13	1,69	0,47
Aps2 3myEGFP	15	88	20	3,84	1,40
Arc18 1myEGFP	15	368	65	4,80	1,27
Ark1 3myEGFP	10	16	19	2,60	1,49
Art3 3myEGFP	14	34	10	1,70	0,39
Bbc1 3myEGFP	10	78	18	1,34	0,35
Bzz1 3myEGFP	22	100	16	1,90	0,34
Cap1 3myeGFP	8	406	133	3,14	0,88
Cap2 3myEGFP	10	420	120	3,60	0,90
Chc1 3myEGFP	5	363	175	0,96	0,13
Crn1 3myEGFP	15	108	27	0,99	0,14
Ede1-3myGFP	35	138	56	0,64	0,15
End3-3myEGFP	21	87	30	2,42	0,96
Ent1 3myEGFP	16	171	28	2,96	0,62
Ent2 3myEGFP	17	183	40	2,60	0,73
Inp51 3myEGFP	7	24	7	1,78	0,47
Inp52 3myEGFP	7	47	20	0,81	0,17
Las17 1myEGFP	28	82	24	2,01	0,51
Lsb3 3myEGFP	13	335	98	1,78	0,64
Myo3 3myEGFP	21	172	61	2,14	0,45
Myo5 3myEGFP	13	152	30	2,58	0,39
Pan1 1myEGFP	52	140	45	2,19	0,71
Rvs161 1myEGFP	23	721	217	3,71	1,52
Rvs167 1myEGFP	32	354	89	3,15	1,07
Scd5 3myEGFP	13	31	6	2,03	0,34
Scp1 3myEGFP	21	163	36	4,18	0,85
Sla1 3myEGFP	13	427	131	1,77	0,28
Sla2-3myEGFP	28	116	39	1,62	0,37
Srv2 3myEGFP	15	162	52	0,48	0,10
Syp1 3myEGFP	12	45	13	1,26	0,24
Vrp1 3myEGFP	9	228	52	1,56	0,34
Yap1801 3myEGFP	20	44	17	2,43	0,82
Yap1802 3myEGFP	24	33	13	1,84	0,74

N denotes the number of single cell measurements. Each single cell measurement is considered to be one experiment. Particle concentration denotes the number of particles measured for 3myeGFP tagged proteins. SD represents the standard deviation.

Table S2 – Overview of previously reported interaction with the corresponding reference

		Reference
Apl1	Apl3	Luo Y1, et al. 2010. J Am Soc Mass Spectrom. 21:34-46
Ede1	Yap1802	Howard JP, et al. 2002. J Cell Biol. 157:315-26
Ede1	Syp1	Reider, A. et al. 2009. EMBO J. 28:3103–16
Ede1	Ent1	Aguilar, R.C. et al. 2003. J. Biol. Chem. 278:10737–43
Ede1	End3	Gavin A.C., et al. 2006. Nature. 440:631-6
Ede1	Sla2	Gavin A.C., et al. 2006. Nature. 440:631-6
Pan1	Yap1801	Wendland B. and Emr S.D. 1998. J Cell Biol. 141:71-84
Pan1	Yap1802	Howard J.P. et al. 2002. J Cell Biol. 1572:315-26
Pan1	Scd5	Zeng, G. et al. 2007. Mol Biol Cell. 18:4885-98
Pan1	End3	Toshima, J. et al. 2007. Mol Biol Cell. 18:658-68
Pan1	Sla2	Toshima, J. et al. 2007. Mol Biol Cell. 18:658-68
Pan1	Sla1	Tang, H.Y. et al. 2000. Mol Cell Biol. 20:12-25
Pan1	Ent1	Aguilar, R.C. et al. 2003. J Biol Chem. 278:10737-43
Sla1	Syp1	Gavin A.C., et al. 2006. Nature. 440:631-6
Sla1	Scd5	Tonikian R. et al. 2009. PLoS Biol. 7:e1000218
Sla1	End3	Tang, H.Y. et al. 2000. Mol Cell Biol. 20:12-25
Sla1	Rvs167	Stamenova, S.D. et al. 2004. J Biol Chem. 279:16017-25
Sla1	Bzz1	Gavin A.C., et al. 2006. Nature. 440:631-6
Sla1	Las17	Michelot, A. et al. 2010. Curr Biol. 20:1890-9
Sla1	Sla2	Gourlay, C.W. et al. 2003. J Cell Sci. 116:2551-64
End3	Yap1802	Howard J.P. et al. 2002. J Cell Biol. 1572:315-26
End3	Scd5	Zeng, G. et al. 2007. Mol Biol Cell. 18:4885-98
End3	Las17	Gavin A.C., et al. 2006. Nature. 440:631-6
End3	Sla2	Gavin A.C., et al. 2006. Nature. 440:631-6
Rvs167	Scd5	Tonikian R. et al. 2009. PLoS Biol. 7:e1000218
Rvs167	Rvs161	Navarro, P. et al. Biochim Biophys Acta. 1343:187-92
Rvs167	Las17	Friesen H. et al. 2003. Mol Biol Cell. 14:3027-40
Las17	Bzz1	Michelot, A. et al. 2010. Curr Biol. 20:1890-9
Las17	Bbc1	Michelot, A. et al. 2010. Curr Biol. 20:1890-9
Las17	Sla2	Gavin A.C., et al. 2006. Nature. 440:631-6
Las17	Rvs161	Michelot, A. et al. 2010. Curr Biol. 20:1890-9
Bzz1	Bbc1	Tonikian R. et al. 2009. PLoS Biol. 7:e1000218
Self		
Apl3		Babu, M. et al. 2012. Nature. 489:585-9*
Ede1		Wang Y. et al. 2012. Mol Biol Cell 23:3911-22
Syp1		Reider, A. et al. 2009. EMBO J. 28:3103–16
Pan1		Miliaras, N.B. et al. 2004. Traffic. 5:963-78.
Sla1		Di Pietro, S.M. et al. 2010. EMBO J. 29:1033-44
Sla2		Yang, S. et al. 1999 Mol Biol Cell. 10:2265-83
Rvs167		Lombardi, R. et al. 2001. J Biol Chem. 276:6016-22
Las17		Madania, A. et al. 1999. Mol Biol Cell. 10:3521-38.
Bbc1		Krogan, N.J. et al. 2006. Nature. 440:637-43

* The initial cut-off by Babu et al. 2012 indicated self-interaction of Apl3. However, further statistical analysis in the same study with a high confidence interaction network did not include the Apl3 self-interaction

Table S3 - Overview of FCCS data presented in this study (corresponding to Fig.2)

	Number of Measurements	Particle concentration green		Particle concentration red		Complex		
	N	nM	SD	nM	SD	nM	SD	Kdeff (nM)
Apl1-Apl1	11	39	16	26	9	No interaction		
Apl1-Apl3	16	47	23	59	23	23	13	37
Apl1-Ede1	6	37	16	96	18	No interaction		
Apl3-Apl3	13	37	13	39	13	No interaction		
Apl3-Ede1	26	71	16	115	37	No interaction		
Bbc1-Bbc1	13	57	16	73	10	19	7	115
Bbc1-Sla1	11	127	41	245	45	No interaction		
Bzz1-Bbc1	15	81	20	121	26	8	3	994
Bzz1-Sla1	17	81	16	211	54	14	10	976
Don1-Don1	17	53	12	46	12	36	10	4
Don1-Ste11	11	43	11	29	6	No interaction		
Ede1-Ede1	28	56	13	47	15	12	8	127
End3-Ede1	3	87	21	102	20	No interaction		
End3-End3	8	85	56	64	27	No interaction		
End3-Sla1	4	36	8	220	46	No interaction		
Ent1-Ede1	11	242	50	101	31	No interaction		
Ent1-Ent1	20	87	18	40	9	No interaction		
Las17-Bbc1	20	100	24	135	24	16	15	631
Las17-Bzz1	19	78	19	74	15	9	4	475
Las17-End3	7	45	25	99	31	8	6	438
Las17-Las17	2	32	9	43	2	No interaction		
Las17-Rvs161	5	54	10	311	23	No interaction		
Las17-Sla1	20	107	38	156	45	32	15	286
Las17-Sla2	7	73	41	84	23	No interaction		
Pan1-End3	34	123	27	105	25	70	18	27
Pan1-Ent1	6	110	61	158	56	No interaction		
Pan1-Pan1	17	73	17	75	21	No interaction		
Pan1-Sla1	9	140	46	163	32	No interaction		
Rvs161-Rvs161	10	197	38	160	21	No interaction		
Rvs167-Rvs161	9	211	40	274	51	196	50	6
Scd5-End3	4	39	13	102	33	No interaction		
Scd5-Pan1	16	51	15	162	39	No interaction		
Scd5-Scd5	22	23	5	40	15	No interaction		
Scd5-Sla1	5	39	11	356	45	No interaction		
Sla1-Sla1	11	361	116	132	40	No interaction		
Sla2-Ede1	11	117	24	100	24	15	3	590
Sla2-End3	3	185	54	85	24	18	6	622
Sla2-Pan1	5	97	21	109	30	14	6	539
Sla2-Sla1	2	202	21	323	0	No interaction		
Sla2-Sla2	19	83	19	58	10	19	5	133
Syp1-Ede1	15	24	6	91	27	7	3	227
Syp1-Sla1	7	16	5	128	21	No interaction		
Syp1-Syp1	13	16	4	17	8	No interaction		
Yap1801-Ede1	13	45	25	96	27	No interaction		
Yap1801-Pan1	5	49	30	164	31	No interaction		
Yap1802-Ede1	12	50	19	119	38	No interaction		
Yap1802-End3	4	34	35	106	16	No interaction		
Yap1802-Pan1	4	33	20	146	41	No interaction		

N denotes the number of single cell measurements. Each single cell measurement is considered to be one experiment. 'Particle concentration GFP' and 'Particle concentration mCherry' denote the number of particles measured for the 3myeGFP tagged proteins and 3mCherry tagged proteins, respectively. Only K_d^{eff}-values for values < 1000 nM are shown. SD represents the standard deviation.

Table S4 – Yeast strains used in this study

Strains generated for FCS/FCCS experiments in Fig. 1, Fig 2, Fig 3B and Fig. S1 were generated in the background strains BY4741 (myeGFP::natNT2 and 3myeGFP::natNT2) and Y8205 (3mCherry::hphNT1) and are not listed in this table.

Strain	Genotype	Source
BY4741	MATa, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0*	Brachmann et al. 1998
Y8205	MAT**α**, *can1Δ ::STE2pr-HIS5, lyp1Δ::STE3pr-LEU2, his3Δ1, leu2Δ0, ura3Δ0*	Tong and Boone 2007
MKY0532	MATa, *his3200, leu2-3,112, ura3-52, lys2-801, yap1801Δ::natNT2, yap1802Δ::natNT2, apl3Δ::natNT2, EDE1-yeGFP::HIS3MX6*	Kaksonen lab
MKY0172	MATa, *his3-Δ200, leu2-3,112, ura3-52, lys2-801, EDE1-yeGFP::HIS3MX6*	Kaksonen lab
MKY2231	MATa, *ura3-52, leu2Δ1, his3Δ200, trp1Δ63, DON1-eGFP::kanMX*	This study
YCM452 (MKY2232)	MATa, *ura3-52, leu2Δ1, his3Δ200, trp1Δ63, natNT2::Pcyc-eGFP-DON1 STE11::3mCherry::hphNT1*	Maeder et al. 2007
YCM449 (MKY2233)	MATa, *ura3-52, leu2Δ1, his3Δ200, trp1Δ63, natNT2::Pcyc::eGFP-DON1::3mCherry::KanMX6*	Maeder et al. 2007
MKY2599	MATa, *his3-Δ200, leu2-3, ura3-52, lys2-801, tor1-1, fpr1::klUra3, ede1$^{\Delta 591-1381}$-FKBP-(24)-myeGFP::kanMX4*	This study
MKY2602	MAT**α**, *his3-Δ200, leu2-3, ura3-52, lys2-801, tor1-1, Fpr1::klUra, ede1$^{\Delta 591-1381}$-FRB-(24)-myeGFP::kanMX4*	This study
MKY2612	MATa/MAT**α**, *his3-Δ200/his3-1, leu2-3/leu2-0, ura3-52/ura3-0, lys2-801/LYS, EDE1-eGFP::HIS3MX6/EDE1-TAP::URA3*	This study
MKY2620	MATa, *his3-1, leu2-0, met15-0, ura3-0, Ede1-TAP::URA3*	This study
MKY2669	MATa, *his3-1, leu2-0, met15-0, ura3-0, ede1$^{\Delta 591-1391}$-myeGFP::natNT2*	This study
MKY2670	MATa, *his3-1, leu2-0, met15-0, ura3-0, ede1$^{\Delta 901-1391}$-myeGFP::natNT2*	This study
MKY2671	MATa/ MAT**α**, *can1Δ::Ste2pr-Leu2/Can1+, lyp1::/Lyp1+, his3-1/his3200, leu2-3/ leu2-0, ura3-52/ura3-0, lys2-801/Lys2+, fpr1::klUra3/ fpr1::klUra3, ede1$^{\Delta 591-1381}$-myeGFP-FRB(24)::kan/ede1$^{\Delta 591-1381}$- myeGFP-FKBP(24)::kanMX4*	This study
MKY1037	MATa, *his3-Δ200 , leu2-3,112, ura3-52, lys2-801, ADH$_{pr}$yeGFP-EDE1::natNT2*	Kaksonen Lab
MKY2812	MAT**α**, *his3-Δ200, leu2-3,112, ura3-52, lys2-801, tor1-1, fpr1Δ, EDE1-myeGFP::natNT2*	This study
MKY0158	MATa, *his3-Δ200, leu2-3,112, ura3-52, lys2-801, EDE1-myeGFP::kanMX4*	Kaksonen lab
MKY2814	MATa, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, ede1$^{-\Delta EH}$-myeGFP::kanMX4*	This study
MKY2994	MATa, *his3-Δ200*, leu2-3,112, ura3-52, lys2-801, tor1-1, fpr1Δ, *ede1Δ:: hphNT1, SYP1-3myeGFP::natNT2*	This study
MKY2997	MATa, *his3-Δ200, leu2-3,112, ura3-52, lys2-801, tor1-1, fpr1Δ, ede1$^{\Delta CC}$, SYP1-3myeGFP::natNT2*	This study
MKY2819	MATa, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, EDE1$_{pr}$1myeGFP::natNT2*	This study
MKY2820	MATa, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, EDE1$_{pr}$3myeGFP::natNT2*	This study
MKY2931	MATa/ MAT**α**, *can1Δ ::STE2pr-HIS5/CAN, lyp1Δ::STE3pr-LEU2/LYP, his3Δ1, leu2Δ0, met15Δ0/MET15+, ura3Δ0, ede1$^{\Delta 591-1381}$-3myeGFP::natNT2 ede1$^{\Delta 591-1381}$-mCherry::hphNT1*	This study
MKY3012	MATa, *his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, ede1^{CC}-myeGFP::kanMX4*	This study

MKY2953	MATa/ MAT**α**, *his3-Δ200, leu2-3,ura3-52, lys2-801, tor1-1, fpr1Δ, ede1$^{\Delta CC}$ -mCherry-FKBP(24)::natNT2/ede1$^{\Delta CC}$ -mCherry-FRB(24)::hphNT1, SYP1-3myeGFP::natNT2*	This study
MKY2823	MATa/ MAT**α**, *his3-Δ200, leu2-3, ura3-52, lys2-801, tor1-1, fpr1Δ:: klUra3, ede1$^{\Delta 591}$ -myeGFP-FKBP(24)::kanMX4/ede1$^{\Delta 591}$-myeGFP-FRB(24)::kanMX4, SLA1-mCherry::hphNT1*	This study

Table S5 - Plasmids used in this study

Name	**Tag**	**Marker**	**Origin**
pST70 (pMK0051)	3mCherry(dcu)	*hphNT1*	This study
pST72 (pMK0053)	3myeGFP(dcu)	*natNT2*	This study
pMK0075	1myEGFP	*natNT2*	This study
pMaM57	1mCherry	*hphNT1*	This study
pGSKU-1	-	*kanMX4/URA*	Storici and Resnick, 2006
pMK0070	myEGFP-FRB(24)	*kanMX4*	Oriol Gallego (Gallego et al. 2013)
pMK0074	myEGFP-FKBP(24)	*kanMX4*	Oriol Gallego (Gallego et al. 2013)
pBS1539	TAP	*URA*	Puig et al. 2001
pKS133	-	*hphNT1*	Janke et al. (2004)
pKS134	-	*natNT2*	Janke et al. (2004)
pFA6-kanMX	-	*kanMX4*	Knop lab
pYM–N9	ADH_{pr} - yeGFP	*natNT2*	Janke et al. (2004)

dcu = different codon usage (for each repeat of the florescent protein)

Supplementary References

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