

**Table S1. Immunoblotting measurements of abundances of key components**

Protein	Name	Before pheromone treatment			After pheromone treatment*				Proteomic study		Catalog or ID number	Dilution	Antibody used for immunoblotting	
		Molecules/cell <sup>†</sup>	SEM	n <sup>‡</sup>	Conc (nM) <sup>§</sup>	Molecules/cell <sup>†</sup>	SEM	n <sup>‡</sup>	Fold increase <sup>§</sup>	Molecules/cell <sup>¶</sup>			Fold difference <sup>  </sup>	Species and source
G protein-coupled receptor**	Ste2	6,622	361	10	380						NA	1:5,000	Rabbit, custom production; Covance <sup>††</sup>	
G-α	Gpa1	2,390	262	6	137	3,299	245	6	1.38	9,920	0.24	SC 6788	1:200	Goat; Santa Cruz Biotechnology
G-β	Ste4	2,045	107	5	117					2,050	1	SC 6785	1:200	Goat; Santa Cruz Biotechnology
PAK kinase	Ste20	4,212	503	7	241					259	16.26	SC 6777	1:200	Goat; Santa Cruz Biotechnology
Scaffold	Ste5	484	61	7	28					1,900	0.25	NA	1:5,000	Rabbit, custom production; Covance <sup>††</sup>
MAPKKK binding partner	Ste50	1,149	109	5	66					1,670	0.69	NA	1:5,000	Rabbit, custom production; Covance <sup>††</sup>
MAPKKK	Ste11	3,524	93	5	202					736	4.79	SC 6769	1:200	Goat; Santa Cruz Biotechnology
MAPKK	Ste7	924	73	6	53					672	1.38	SC 6770	1:200	Goat; Santa Cruz Biotechnology
MAPK	Fus3	20,413	2,855	5	1,169	26,743	2,973	5	1.31	8,480	2.41	SC 6773	1:200	Goat; Santa Cruz Biotechnology
MAPK	Kss1	20,795	2,296	4	1,190					5,480	3.79	SC 6775	1:200	Goat; Santa Cruz Biotechnology
MAPK phosphatase	Msg5	38	3	9	2	69	4	9	1.82	538	0.07	NA	1:5,000	Rabbit, custom production; Covance <sup>††</sup>
Transcriptional activator	Ste12	1,390	44	6	80	1,819	103	6	1.31	1,920	0.72	SC 6798	1:200	Goat; Santa Cruz Biotechnology
Transcriptional repressor	Dig1	4,799	453	6	275					1,460	3.29	NA	1:5,000	Rabbit, custom production; Covance <sup>††</sup>
Transcriptional repressor	Dig2	1,184	84	9	68					1,310	0.9	NA	1:5,000	Rabbit, custom production; Covance <sup>††</sup>
Cell cycle inhibitor	Far1	245	16	5	14	502	23	5	2.05	238	1.03	SC 6791	1:200	Goat; Santa Cruz Biotechnology
Scaffold/MAPKK	Pbs2	2,459	166	5	141					2,160	1.14	SC 6813	1:200	Goat; Santa Cruz Biotechnology
MAPK	Hog1	5,998	396	5	343					6,780	0.88	SC 6815	1:200	Goat; Santa Cruz Biotechnology

\*Cells were treated with 1 mM α-factor pheromone for 15 min.

†Average of n experiments.

‡Concentration if protein was uniformly distributed in a cell volume of 29 fL.

§Abundance after 15-min pheromone treatment divided by abundance without pheromone.

¶Ghaemmaghami et al. (1).

||Abundance measured in this study divided by the abundance measured in the study by Ghaemmaghami et al. (1).

\*\*Measurements performed by others (2–11).

††M. Maxon and I. Herskowitz, unpublished data.

‡‡Pincus et al. (12).

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