Supporting Information

Protein expression patterns of the yeast mating response

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1, mating pathway relative proteins (156 proteins).

Protein name	R value	Protein name	R value	Protein name	R value	Protein name	R value	Protein name	R value	Protein name	R value
YIL083C	1.76	GLC7	0.44	SNF5	0.28	PYC1	0.14	FAR1	-0.04	RIM101	-0.25
MFA1	1.22	RCS1	0.43	SIN3	0.27	STB5	0.13	SNF2	-0.06	VPS27	-0.25
TEC1	1.22	TOF1	0.41	PMI40	0.27	SAS3	0.13	CLN2	-0.06	CIK1	-0.27
KSS1	1.21	CLK1	0.40	KIN1	0.27	MRPL3	0.11	RPL23A	-0.06	SWI3	-0.30
UME6	1.10	MPT5	0.40	PDC1	0.26	HSP82	0.10	YPS1	-0.07	CYK2	-0.30
CAC2	0.93	TYE7	0.40	CRZ1	0.26	YCL056C	0.10	FUS1	-0.07	DFG5	-0.32
ECM18	0.86	SFP1	0.40	CHA4	0.26	SIR2	0.09	MET18	-0.09	GPA1	-0.33
PPH3	0.84	YAR027W	0.39	JJJ2	0.26	AIM44	0.08	NOT3	-0.11	RPA49	-0.34
CSE4	0.83	YFL004W	0.39	YPL146C	0.26	APA2	0.08	YLR194C	-0.11	FKH2	-0.38
ZAP1	0.75	SST2	0.38	YNL279W	0.25	RSR1	0.08	SKO1	-0.11	YKL185W	-0.45
YMR204C	0.73	MRPL24	0.37	SCW11	0.25	STE6	0.07	DBR1,	-0.12	SSU81	-0.45
ASN1	0.73	CPR6	0.37	YOR246C	0.25	AFIL	0.06	SIN4	-0.12	HIR3	-0.47
CRH1	0.69	SET2	0.36	STE12	0.25	CDC39	0.06	SNF1	-0.13	DOT6	-0.51
STE11	0.67	YPK2	0.35	TBF1	0.24	SLT2	0.06	SAM1	-0.13	PRP39	-0.55
YLL013C	0.67	YKR089C	0.35	CDC28	0.24	MSS11	0.06	RPS1B	-0.13	MIG1	-0.61
FKH1	0.66	WHI2	0.34	YGL157W	0.23	RTF1	0.05	CDC11	-0.14	AGA1	-0.62
CAF4	0.61	GYP7	0.33	BDF2	0.23	BOP3	0.04	TEF1	-0.14	STE2	-0.65
YHB1	0.60	MRPS35	0.33	IDH2	0.22	PPQ1	0.02	YPL192C	-0.15	RAD51	-0.69
KSP1	0.54	OPY2	0.32	FUS2	0.22	RTG2	0.00	ISW1	-0.15	RLM1	-0.70
KAR3	0.51	EDE1	0.32	SKN7	0.21	AZF1	0.00	YIL037C	-0.15	SSF2	-0.73
YJL107C	0.50	UTH1	0.31	AXL1	0.20	MET2	-0.01	MDS3	-0.18	YKL222C	-0.74
YOR248W	0.48	MPK1	0.31	SRL1	0.18	FUN30	-0.02	AMN1	-0.18	MSI1	-0.87

KAR9	0.47	RIC1	0.31	YOR086C	0.18	HAL9	-0.02	PHD1	-0.20	BEM4	-0.87
CTF4	0.47	YJU2	0.31	ARG81	0.18	YPR004C	-0.02	THI3	-0.21	HCM1	-0.91
YKR077W	0.45	YOR385W	0.29	NDD1	0.16	GAL11	-0.03	AKR1	-0.24	SPT10	-0.95
YNL274C	0.45	SEN2	0.28	NPT1	0.15	PDC2	-0.03	SET1	-0.25	LEU3	-1.52

Table S1. The names of all 156 proteins.

2. Device design, fabrication and operation

Our designed microfluidic chip can be divided into two identical parts. Each part contains 48 cell channels and two solution inlets. Different strains would be loaded to different channels from 48 cell inlets and culture solution injected from two solution inlets would provide same cellular environment for 48 yeast strains. We designed necks in every channel to block yeast cells from going into other channels but these necks would not block culture solution. In every channel, we designed observation chamber the height of which is close to cell size and can fix yeast cells well. This is helpful for time-series observation by microscope.

Our microfluidic chip was fabricated with PDMS (polydimethylsiloxane, RTV615, Momentive Performance Materials Inc.) using standard soft lithography technology. The mould of our microfluidic chip was constructed by applying photoresist (SU-8, MicroChem Corp, USA) to a silicon wafer using a standard multi-layer overlay method. The mould has three layers with three different heights. The necks at the end of the observation chambers have a height of approximately 2 μm (green parts in Figure 2c). The height of the 96 observation chambers is approximately 4 μm (yellow part in Figure 2c). The height of the main channels for loading cells and culture inflow is much greater than 4 μm (red parts in Figure 2c). We first fabricated the lowest layer of our mould (the necks) on the silicon wafer. The silicon wafer coated with SU-8 3002 was spun to a thickness approximately 2 μm using a spin coater (Laurell Technologies Corporation, Ws-650 MZ-23NPP, USA). Then, we prebaked the silicon wafer on a hot plate at 95℃ for 2 minutes, followed by exposure to 365 nm UV light with a power of 3.7 mW/cm2 for 28 seconds. Next, the

silicon wafer was postbaked on a plate at 95℃ for 2 minutes. After being cooled, the wafer was immersed into developing solution for another 20 seconds. Consequently, we obtained 2 μm mould on the wafer. We then fabricated a second layer with a height of 4 μm using a similar procedure. We coated the previous silicon wafer with SU-8 3003, which was spun to a thickness of approximately 4 μm. After 2 minutes of prebaking, 30 seconds of exposure to UV light, 2 minutes of postbaking and 30 seconds of development procedures, we obtained a second layer on the wafer. We then fabricated the third layer on the same silicon wafer using a similar procedure. Finally, we obtained the three-layer mould for our microfluidic chip.

To fabricate the microfluidic chip, we firstly mixed approximately 26.4 g of PDMS monomer and 3.3 g of curing agent together. The mixture was then poured over the mould followed by a degassing procedure. After curing at 70℃ for 40 minutes, the cured PDMS with a thickness approximately 0.5 cm was peeled off from the mould. We then punched holes for the loading wells and the inlets of the culture solution. After all the holes were punched, we bonded the PDMS to a 4 cm ╳ 8 cm glass slide using air plasma. This step completed the fabrication of the microfluidic chip.

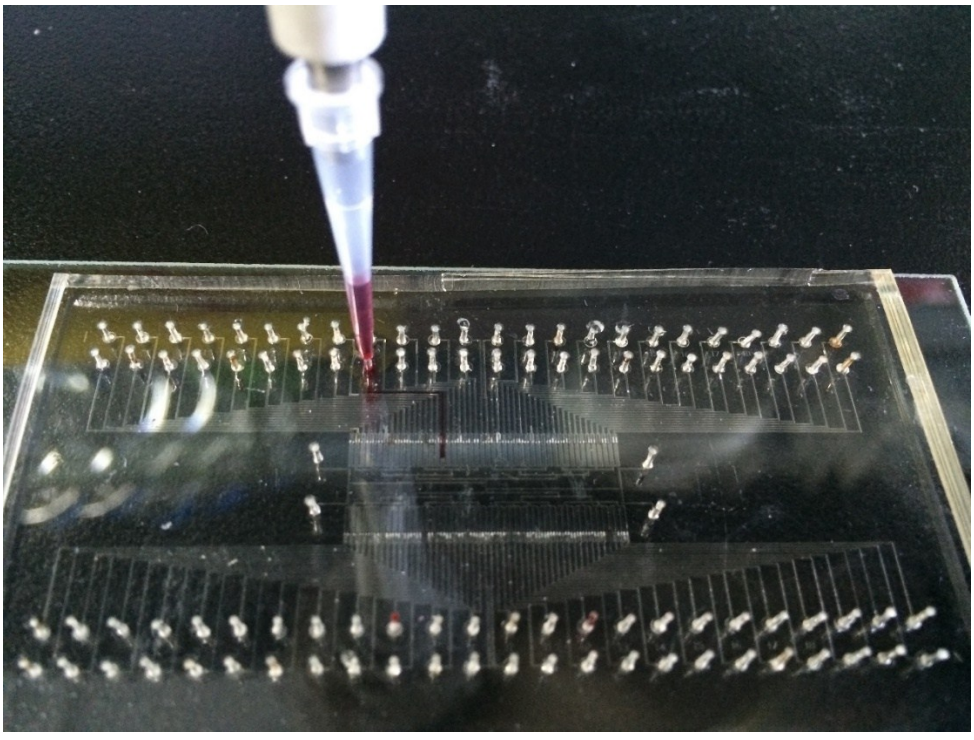

Fig S1 Image of loading cells into the chip

For operation, we first degassed the microfluidic chip using a vacuum pump for approximately 15 minutes. Then, we loaded cells into all the channels using a 10 μl pipette. In detail, we loaded 10 μl of cell liquid into a pipette, inserted the tip into the cell inlets because the diameter of the cell inlets is close to the size of 10 μl tips, and then pushed the cell liquid into the channels (Fig. S1). After loading all 96 strains on the chip, we prepared four 5 ml injection syringes, two of which contained 5 ml of normal culture solution. The third injection syringe contained 5 ml of culture solution with high-dose alpha-factor, and the last injection syringe contained 5 ml of culture solution with low-dose alpha-factor. We then connected the four injection syringes to four PE tubes, and the other ends of tubes were inserted into the four inlets in the chip. We then put our microfluidic chip in a microscope system, selected observation positions and began the automatic microscopy image capture using the microscope control software. At the beginning, we injected normal culture solution from one of the solution inlets through a syringe pump with a flow speed of 400 μl/h. After the injection of normal culture solution for approximately 1 hour, we injected culture solution containing alpha-factor from another solution inlet and stopped the normal injection until the end of the experiment. The waste liquid flowed out from the cell loading holes and evaporated from the filter paper that was used to cover the outlets. In a relatively short time (less than 12 hours), the waste liquid could be completely absorbed by the filter paper, thus, we did not have to connect 96 outlet and tubes.

3. The rate of increase in the area of single cells.

We use the rate of increase in the cell area as a rough representation of the cell growth rate. We calculated the rate of increase in the area of different cells in one chamber by differencing the mask pictures using ImageJ software. We found that on average, the rate of increase in the area decreases after alpha-factor treatment (See Fig S2). For example, we examined the cells in two chambers, and the average rates of increase in the area in the two chambers are similar (See Fig S2).

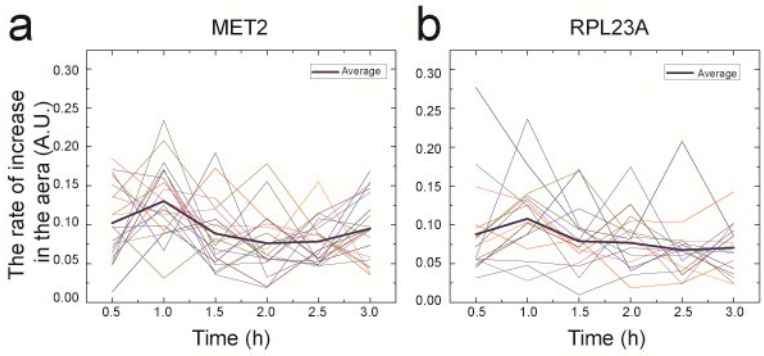

Fig S2 The rate of increase in the area of single cells in two chambers (a, b). The heavy line represents the average rate of increase in the area. We calculated the rate of increase in the area using the equation $\frac{area(t)}{area(t-0.5)}-1$. The time interval is 0.5 h here. We injected high-dose alpha-factor at t=1 h.

4. Variability measurement of two typical protein expression for example.

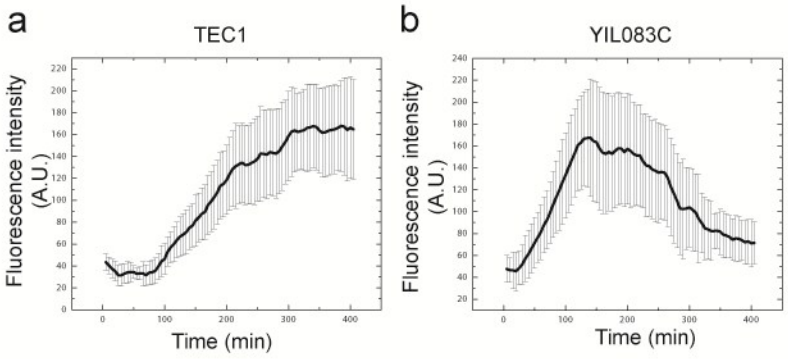

Fig S3 Two protein expression curves with error bars as example. The heavy line represents the average GFP concentration of the cells in the chambers. The error bars represent the standard deviation of the cellular GFP concentration. (a) The protein TEC1 is up-regulated under the high-dose alpha-factor condition. Alpha-factor is injected at t=0. (b) The protein YIL083C is up-regulated first and then adapts under the high-dose alpha-factor condition. Alpha-factor is injected at t=0.

5. The cluster number of some proteins

Protein Name	Cluster Number	Protein Name	Cluster Number	Protein Name	Cluster Number	Protein Name	Cluster Number
SIN4	1	GPA1	1	YOR385W	3	YNL274C	3
YKL222C	1	RIC1	1	YLR194C	3	FUN30	3
SWI3	1	PYC1	1	KAR9	3	TEC1	3

CLN2	1	UTH1	1	FUS1	3	STE11	3
PMI40	1	PDC2	1	STE6	3	SEN2	3
YGL157W	1	RSR1	1	AKR1	3	SLT2	3
HSP82	1	YNL279W	1	SIN3	3	YHB1	3
NPT1	1	SSU81	1	YJU2	3	FAR1	3
MPT5	1	SPT10	2	RPL23A	3	PPH3	3
HIR3	1	THI3	2	ISW1	3	MSI1	3
ARG81	1	NDD1	2	YFL004W	3	TYE7	3
UME6	1	DOT6	2	KIN1	3	PDC1	3
SET2	1	CHA4	2	WHI2	3	IDH2	3
ZAP1	1	RIM101	2	YOR248W	3	ECM18	3
GAL11	1	HCM1	2	FKH1	3	EDE1	3
RTG2	1	YOR246C	2	SAM1	3	MPK1	3
SKN7	1	AXL1	2	KSP1	3	ASN1	3
SFP1	1	AZF1	2	MET2	3	CLK1	3
TBF1	1	STE2	2	AMN1	3	AFIL	3
RTF1	1	CIK1	2	MET18	3	MRPL3	3
BDF2	1	STE12	2	HAL9	3	GYP7	3
RCS1	1	MIG1	2	YIL037C	3	AGA1	3
CAF4	1	LEU3	2	YLL013C	3	SST2	3
CRZ1	1	YKR077W	2	MRPL24	3	CTF4	3
SKO1	1	RPS1B	2	KAR3	3	SNF2	3
SNF1	1	SET1	2	KSS1	3	MDS3	3
BOP3	1	PRP39	2	YMR204C	3	MSS11	3
TOF1	1	FKH2	2	CAC2	3	CYK2	3
PPQ1	1	YAR027W	2	MFA1	3	CPR6	3
YPL146C	1	YPL192C	2	CSE4	3	AIM44	3
CDC39	1	RLM1	2	YCL056C	3	MRPS35	3
YOR086C	1	SAS3	2	GLC7	3	DFG5	3
RPA49	1	TEF1	2	YKR089C	3	SSF2	3
STB5	1	YKL185W	2	OPY2	3	DBR1	3
YPR004C	1	NOT3	2	CRH1	3	YPS1	3
YIL083C	1	CDC11	3	SCW11	3	CDC28	3

Table S2. The cluster number of some proteins.