Supplementary Information:

Quantitative and Dynamic Assay of Single Cell Chemotaxis

Sung Sik Lee\textsuperscript{a,b}, Peter Horvath\textsuperscript{c}, Serge Pelet\textsuperscript{a,b}, Björn Hegemann\textsuperscript{a},
Luke P. Lee\textsuperscript{*d}, and Matthias Peter\textsuperscript{$\S$}\textsuperscript{a,b}

\textsuperscript{a} Institute of Biochemistry, ETH Zurich, Zurich, CH 8093, Switzerland
\textsuperscript{b} Competence Center for Systems Physiology and Metabolic Disease (CC-SPMD), Zurich, CH 8093, Switzerland
\textsuperscript{c} Light Microscopy Center (LMC), Department of Biology, ETH Zurich, Zurich, CH 8093, Switzerland
\textsuperscript{d} Berkeley Sensor & Actuator Center, Department of Bioengineering, University of California, Berkeley, CA 94720-1762, United States

Co-correspondences:
* lplee@berkeley.edu; Tel: +1-510-642-5855, Fax: +1-510-642-5835
$\S$ matthias.peter@bc.biol.ethz.ch; Tel: +41-44-633-6586, Fax: +41-44-633-1298
**Figure S1:** Conventional method for creating an α-factor gradient. α-factor (red drop) solution is continuously dropped on the agarose cell culture pad. This method is limited from high resolution fluorescent imaging because the thick and opaque cell culture pad interferes with fluorescent light and a long-working distance objective lens is needed.
**Figure S2: Integrated platform for quantitative and dynamic assay of single cell chemotaxis.** The automated microfluidic platform can regulate a morphogenetic gradient. By a triggering signal from the computer to the air pressure controller at any given time, high and low concentrations of α-factor are equally provided to the cell culture chamber. Thus, the gradient is established by diffusion of high and low α-factor concentration. The fluidic resistances near the solution reservoirs are designed to tune the flow rate and external fluctuation when air pressure (ΔP) is applied to the reservoirs. The dynamics of budding yeast polarization are multidimensionally recorded including positions, fluorescent channels and time. Images of single yeast cells are segmented, and their morphology and subcellular polarization are quantified. The resulting data sets serve as a basis to validate and improve mathematical models describing this dynamic process.
**Figure S3: Microfluidic chip and assembly.** Overview of the microfluidic chip design (A) Microfluidic chip with feeder wells and pipette tip for cell loading. (B) There are two liquid feeder wells, which may contain varying concentrations of the diffusible substance. A micropipette tip was inserted in the cell inlet hole to introduce (20-100 µL) of the cell suspension to the microfluidic cell culture chamber by gravity flow. After loading, the tip was carefully removed and the cells in microfluidic chamber were examined. (C) Assembly of set-up: the compressed airflow was established by the diaphragm pump, the compressed airtank, the electronic pressure regulator, and the solenoid valves. The airflow was delivered to tow liquid feeder wells.
**Figure S4: Time-lapse images of yeast cells.** Cells grow and continuously make buds without α-factor (A; budding). With uniform α-factor, cells form elongated shapes termed shmoos (B; shmooring). The direction of shmooring is random since α-factor is uniformly distributed and thus binds receptors over the entire cell surface with the same probability. Cells were engineered to express a mating-specific reporter, based on the *FIG1* promoter driving the expression of quadruple-Venus fluorescent protein (*pFIG1*-qV). In response to α-factor, the cells thus express YFP (green), implying that the yeast cells are able to grow and efficiently respond to pheromones in the cell culture chamber for extended periods of time.
Supplementary Table S1: Yeast strains used in this study.

<table>
<thead>
<tr>
<th>Strain No</th>
<th>Relevant genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG123</td>
<td>HMLa HMRa; met ade, ura; EG123 pFIG1-quadVenus::LEU2</td>
<td>Valtz et al., 1995</td>
</tr>
<tr>
<td>ySP107</td>
<td>EG123 pFIG1-quadVenus::LEU2</td>
<td>this study</td>
</tr>
<tr>
<td>yMP315</td>
<td>EG123 far1-s (H7)</td>
<td>Valtz et al., 1995</td>
</tr>
<tr>
<td>BY4741</td>
<td>MATa ura3Δ0; leu2Δ0; his3Δ1; met15Δ0</td>
<td>Openbiosystems</td>
</tr>
<tr>
<td>yBH200</td>
<td>BY4741 CDC24::CDC24-quadVenus; FAR1::FAR1-HA-2xStrp-G861A-HIS3; pRPS2-TMDmCherry::LEU2</td>
<td>this study</td>
</tr>
</tbody>
</table>

**Supplementary Movie S1**

**Dynamics of yeast polarization under in the microfluidic device in response to an α-factor gradient**

The yeast cells polarize towards higher concentrations of mating pheromone (α-factor; blue). The cells express quadruple-Venus fluorescent protein under the control of the mating-specific FIG1 promoter (green).

**Reference**