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**Supplemental Information Figure 1. Related to Figure 3. 21 fitted pAkt distributions and 3 fitted sEGFR distributions used in parameter inference for synthetic data; parametrization 1: Wild type.** We show all fitted single cell distributions (pAkt fits in red, sEGFR fits in blue, *in silico* experimental data in black) used in the inference.

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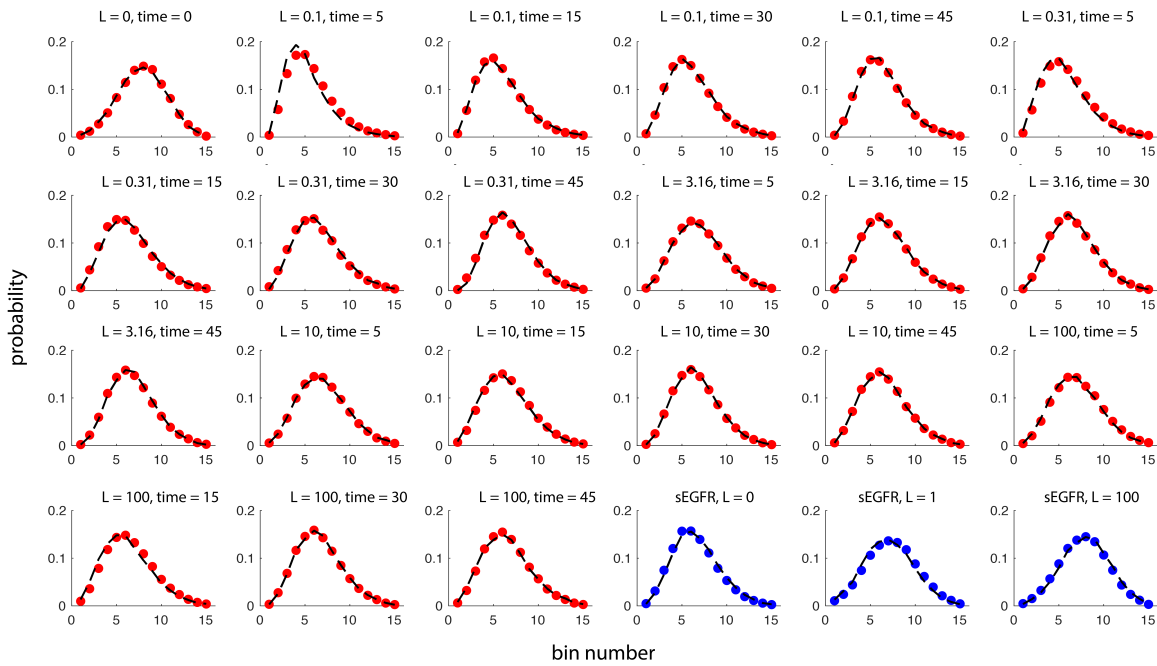14

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**Supplemental Information Figure 2. Related to Figure 3. 21 fitted pAkt distributions and 3 fitted sEGFR distributions used in parameter inference for synthetic data; parametrization 2: EGFR overexpression.** We show all fitted single cell distributions (pAkt fits in red, sEGFR fits in blue, *in silico* experimental data in black) used in the inference.
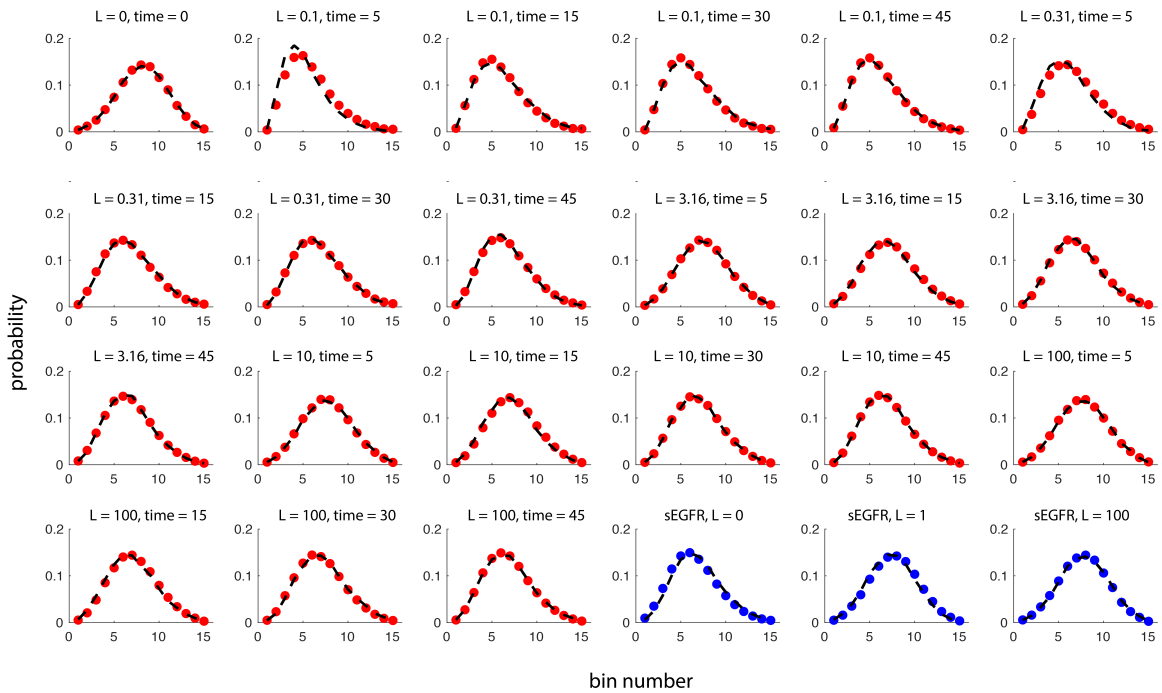
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The plots in the figure are titled (row by row):

Row 1: L = 0, time = 0 | L = 0.1, time = 5 | L = 0.1, time = 15 | L = 0.1, time = 30 | L = 0.1, time = 45 | L = 0.31, time = 5

Row 2: L = 0.31, time = 15 | L = 0.31, time = 30 | L = 0.31, time = 45 | L = 3.16, time = 5 | L = 3.16, time = 15 | L = 3.16, time = 30

Row 3: L = 3.16, time = 45 | L = 10, time = 5 | L = 10, time = 15 | L = 10, time = 30 | L = 10, time = 45 | L = 100, time = 5

Row 4: L = 100, time = 15 | L = 100, time = 30 | L = 100, time = 45 | sEGFR, L = 0 | sEGFR, L = 1 | sEGFR, L = 100

Y-axis: probability

X-axis: bin number

**Supplemental Information Figure 3. Related to Figure 3. 21 fitted pAkt distributions and 3 fitted sEGFR distributions used in parameter inference for synthetic data; parametrization 3: PTEN loss.** We show all fitted single cell distributions (pAkt fits in red, sEGFR fits in blue, *in silico* experimental data in black) used in the inference.
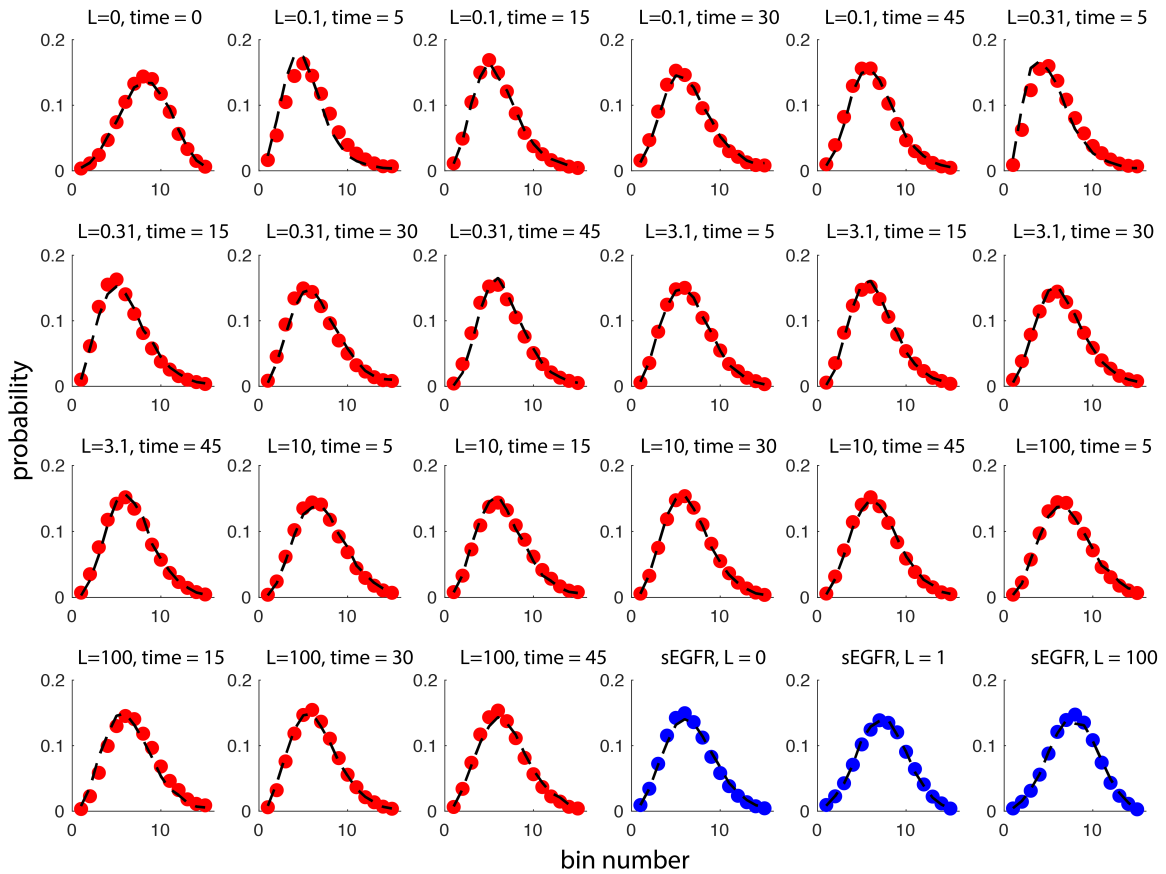
**Supplemental Information Figure 4. Related to Figure 3. 21 fitted pAkt distributions and 3 fitted sEGFR distributions used in parameter inference for synthetic data; parametrization 4: PTEN loss.** We show all fitted single cell distributions (pAkt fits in red, sEGFR fits in blue, *in silico* experimental data in black) used in the inference.

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**Supplemental Information Figure 5. Related to Figure 3. 21 fitted pAkt distributions and 3 fitted sEGFR distributions used in parameter inference for synthetic data; parametrization 5: PTEN loss.** We show all fitted single cell distributions (pAkt fits in red, sEGFR fits in blue, *in silico* experimental data in black) used in the inference.
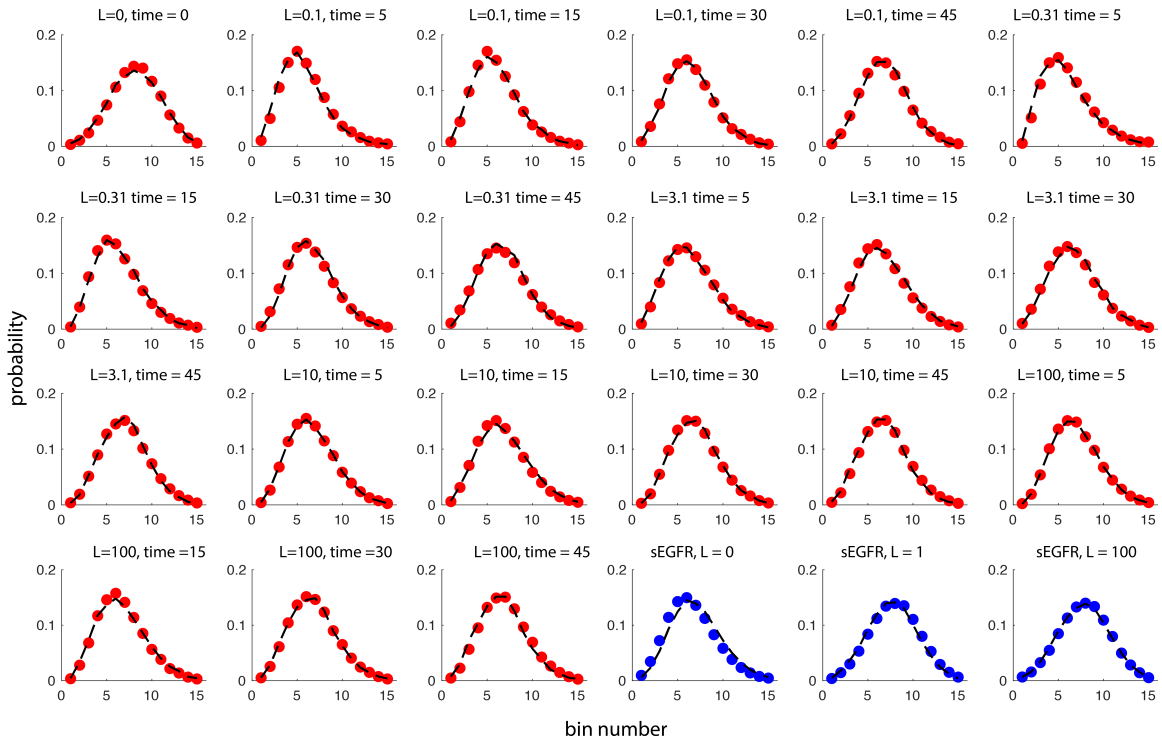
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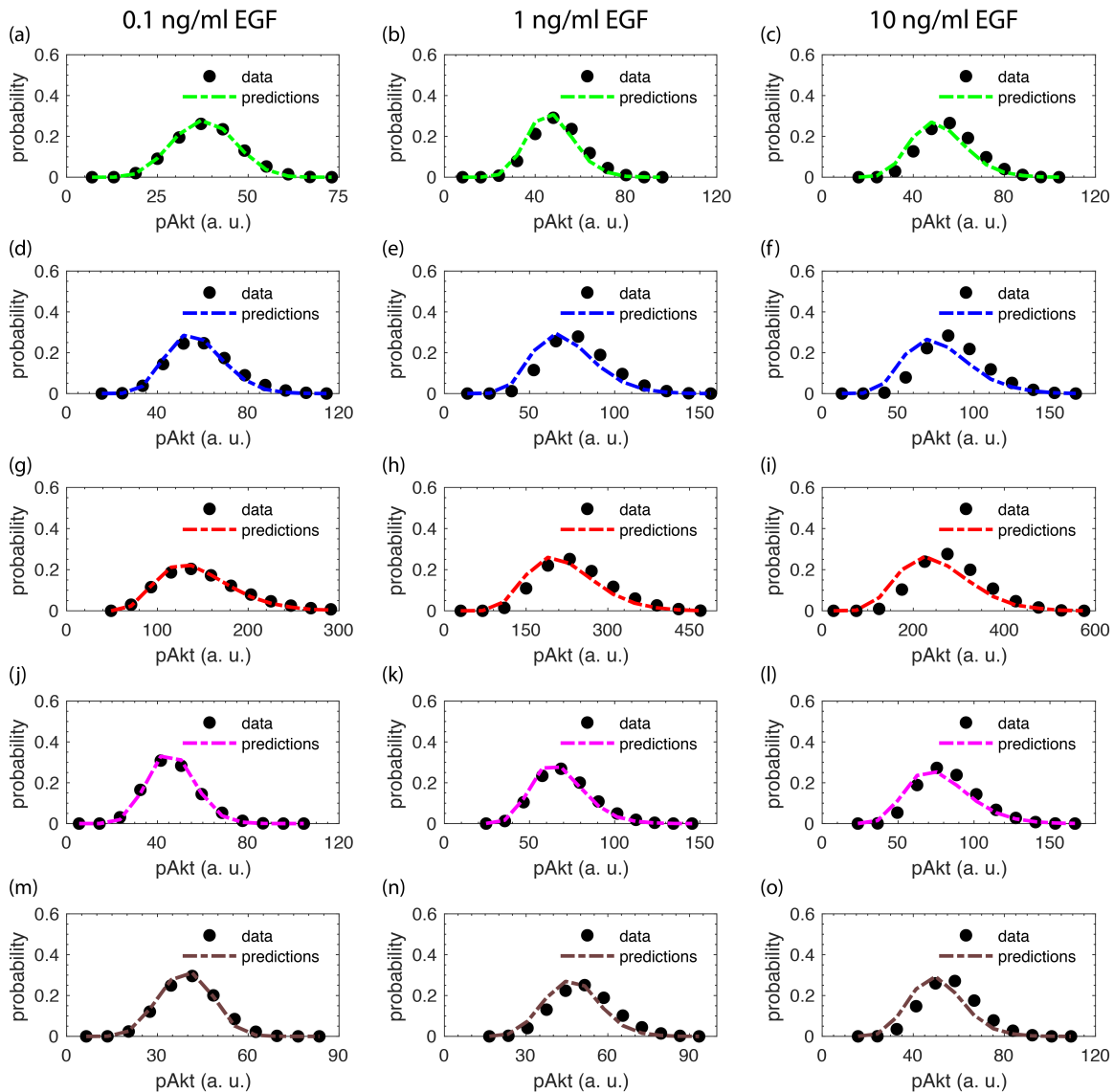
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**Supplemental Information Figure 6. Related to Figure 3. Prediction pAkt levels at late times for different perturbations to the EGFR pathway**. Comparison between single cell heterogeneity in steady state pAkt levels (3 hours of continuous EGF stimulation) as observed in *in silico* data (black circles) and MERIDIAN based predictions (dashed lines) in the "wild type" parametrization of the EGFR pathway (first row, green, a: 0.1 ng/ml EGF, b: 1 ng/ml EGF, c: 10 ng/ml EGF), a parametrization with two-fold overexpression of EGFR (second row, blue, d: 0.1 ng/ml EGF, e: 1 ng/ml EGF, f: 10 ng/ml EGF), a parametrization with a ten-fold decrease in Akt dephosphorylation (third row, red, g: 0.1 ng/ml EGF, h: 1 ng/ml EGF, i: 10 ng/ml EGF), a parametrization with a two-fold decrease in EGFR endocytosis rate (fourth row, pink, j: 0.1 ng/ml EGF, k: 1 ng/ml EGF, l: 10 ng/ml EGF), a parametrization with a two-fold decrease in EGFR dephosphorylation (third row, brown, m: 0.1 ng/ml EGF, n: 1 ng/ml EGF, o: 10 ng/ml EGF).

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bin number
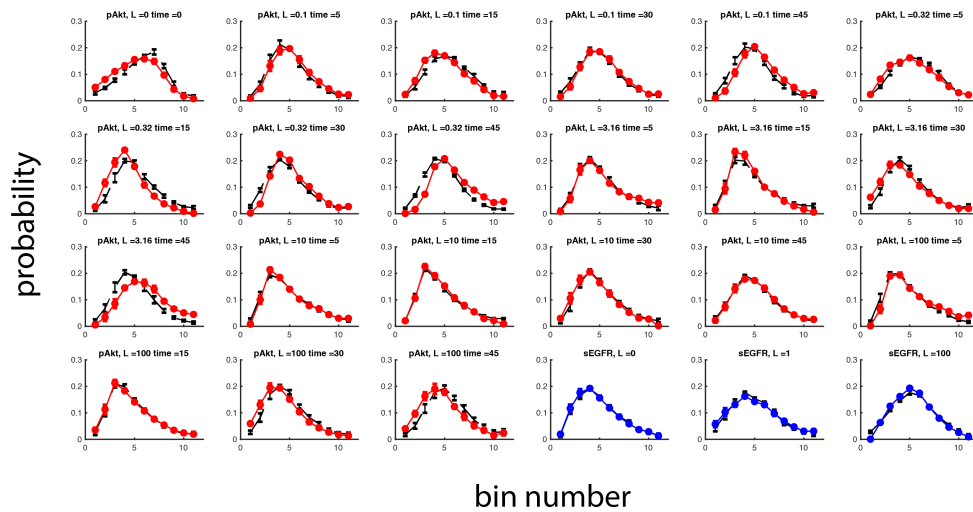
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106 **Supplemental Information Figure 7. Related to Figure 3. 21 fitted pAkt**
107 **distributions and 3 fitted sEGFR distributions used in parameter inference.** We
108 show all fitted single cell distributions (pAkt fits in red, sEGFR fits in blue, experimental
109 data in black) used in the inference. Error bars represent experimentally estimated
110 standard errors in the mean and model estimated uncertainties.
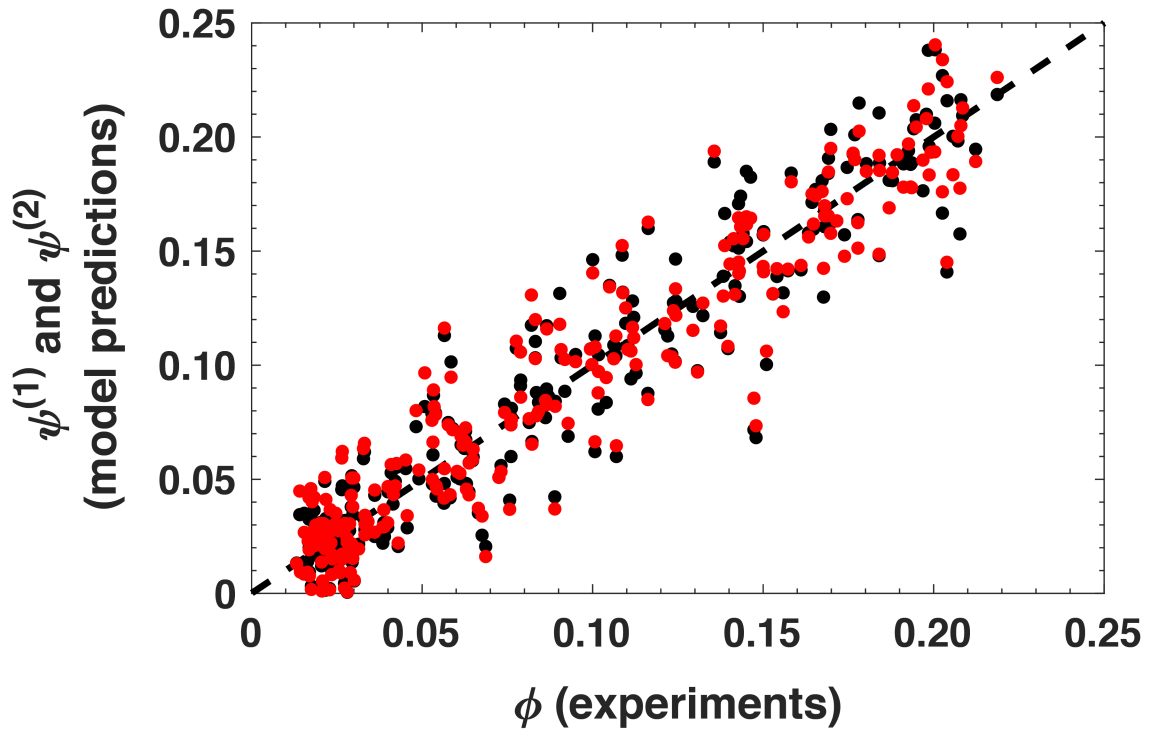
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**Supplemental Information Figure 8. Related to Figure 3. Model predictions agree for two independent calculations.** The correlation between experimentally estimated bin fractions (x axis) and predicted bin fractions (y axis) for two independent searches for the Lagrange multipliers (red and blue dots).
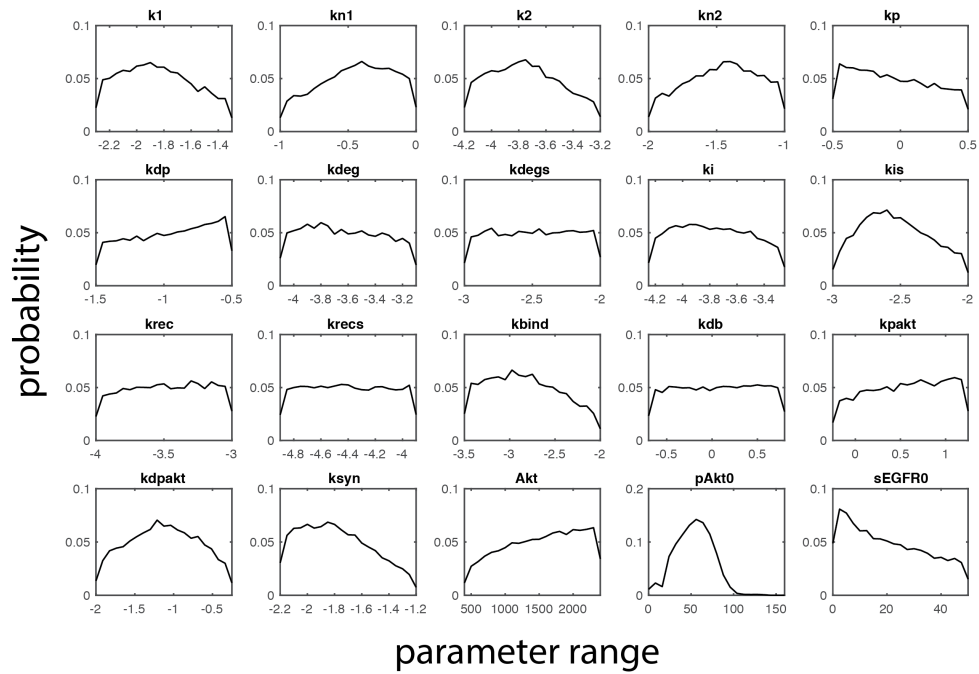
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143  **Supplemental Information Figure 9. Related to Figure 3. Inferred marginal**
144  **distributions of all 20 model parameters. Parameters #1 to #17 are on a $log_{10}$ scale.**

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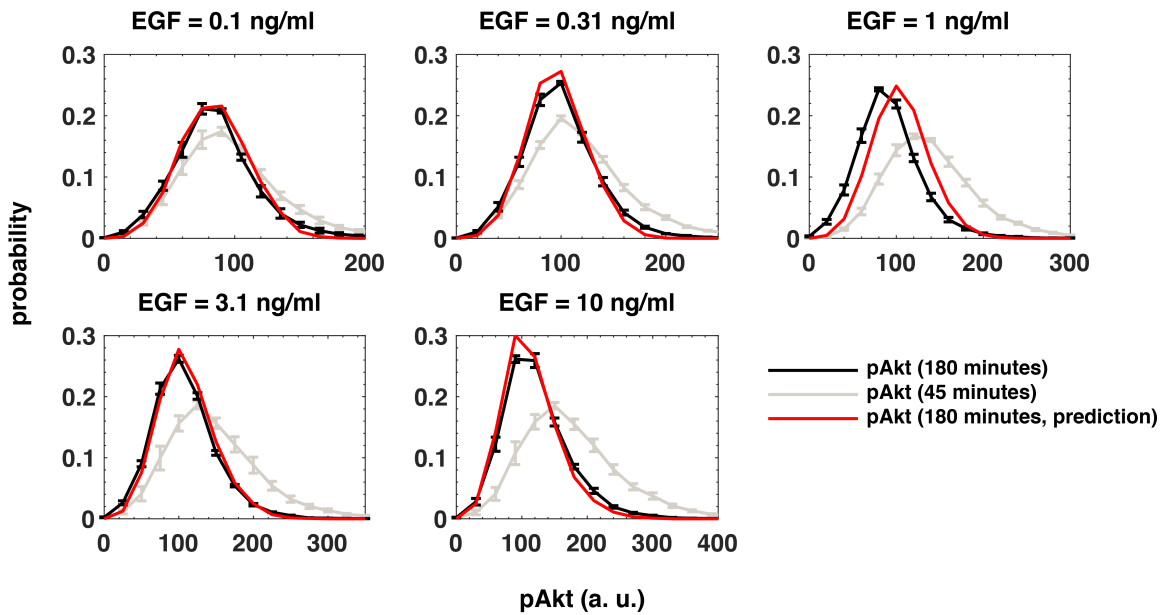
**Supplemental Information Figure 10. Related to Figure 4. Population dynamics has not reached steady state at 45 minutes.** We plot single cell distributions of pAkt levels at 45 minutes (gray lines) and at 180 minutes (experiments in black lines, model fit in red lines) across a broad range of EGF doses. Error bars represent experimentally estimated standard errors in the mean. For simplicity, we do not show the model estimated uncertainties.

| Rate description (and references) | Variable | lower bound (log 10) | upper bound (log 10) | Units |
|---|---|---|---|---|
| EGF binding to EGFR (Schoeberl, Eichler-Jonsson et al. 2002) | $k_1$ | -2.3 | -1.3 | $(ng/ml)^{-1} sec^{-1}$ |
| EGF unbinding from EGFR (Chen, Schoeberl et al. 2009) | $k_{-1}$ | -1 | 0 | $sec^{-1}$ |
| EGF-EGFR and EGFR dimerization (Chen, Schoeberl et al. 2009) | $k_2$ | -4.2 | -3.2 | a. u. |
| EGF-EGFR-EGFR undimerization (Chen, Schoeberl et al. 2009) | $k_{-2}$ | -2 | -1 | $sec^{-1}$ |
| EGFR dimer phosphorylation (Chen, Schoeberl et al. 2009) | $k_{ap}$ | -0.5 | 0.5 | a. u. |
| p-EGFR dephosphorylation (Kleiman, Maiwald et al. 2011) | $k_{dp}$ | -1.5 | -0.5 | $sec^{-1}$ |
| degradation of active EGFRs (Hendriks, Wiley et al. 2003) | $k_{deg}^{*}$ | -3 | -2 | $sec^{-1}$ |
| degradation of inactive EGFRs (Shankaran, Zhang et al. 2012) | $k_{deg}$ | -4.1 | -3.1 | $sec^{-1}$ |
| EGFR delivery to the membrane | $k_{syn}$ | -2.2 | -1.2 | a. u. |
| Internalization of inactive EGFRs (Wiley, Herbst et al. 1991) | $k_i$ | -4.25 | -3.25 | $sec^{-1}$ |
| Recycling rate of inactive EGFR (Herbst, Opresko et al. 1994) | $k_{rec}$ | -4 | -3 | $sec^{-1}$ |
| Internalization of active EGFRs (Wiley, Herbst et al. 1991) | $k_i^{*}$ | -3 | -2 | $sec^{-1}$ |
| Recycling rate of active EGFRs (Herbst, Opresko et al. 1994) | $k_{rec}^{*}$ | -4.9 | -3.9 | $sec^{-1}$ |
| effective rate of p-EGFR binding to Akt | $k_{bind}$ | -3.5 | -2 | a. u. |
| effective rate of p-EGFR unbinding from pEGFR-Akt | $k_{db}$ | -0.7 | 0.8 | $sec^{-1}$ |
| Rate of pAkt dephosphorylation | $k_p$ | -2 | -0.5 | $sec^{-1}$ |
| Rate of Akt phosphorylation | $k_a$ | -0.25 | 1.25 | $sec^{-1}$ |
| Total Akt abundance (not log) | Akt | 400 | 2400 | a. u. |
| Background fluorescence in pAkt (not log) | $b_0$ | 0 | 160 | a.u. |
| Background fluorescence in sEGFR (not log) | $s_0$ | 0 | 50 | a.u. |

**Supplemental Information Table 1. Names of parameters and their ranges. Related to Figure 2, 3, and 4.**

| Species name | Species symbol |
|---|---|
| Free EGF | $u$ |
| Free EGFR monomer | $R$ |
| Internalized free EGFR monomer | $R_i$ |
| EGF bound EGFR monomer | $B$ |
| Internalized EGF bound EGFR monomer | $B_i$ |
| 1 EGF bound EGFR dimer | $D_1$ |
| Internalized 1 EGF bound EGFR dimer | $D_{1i}$ |
| 2 EGF bound EGFR dimer | $D_2$ |
| Internalized 2 EGF bound EGFR dimer | $D_{2i}$ |
| Phosphorylated 1 EGF bound EGFR dimer | $P_1$ |
| Internalized phosphorylated 1 EGF bound EGFR dimer | $P_{1i}$ |
| Phosphorylated 2 EGF bound EGFR dimer | $P_2$ |
| Internalized phosphorylated 2 EGF bound EGFR dimer | $P_{2i}$ |
| Unphosphorylated Akt | $Akt$ |
| Phosphorylated Akt | $pAkt$ |
| P1 bound to Akt | $P_{1Akt}$ |
| P2 bound to Akt | $P_{2Akt}$ |

185 **Supplemental Information Table 2**. **Variables names**. Related to Figure 2, 3, and 4.

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198 **Supplemental Information Tables 3, 4, and 5 are big and are attached as separate**
199 **files.**

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| Parameter | Correlation |
|---|---|
| | |
| 'k1　' | -0.1794 |
| 'kn1 ' | 0.1565 |
| 'k2　' | -0.1888 |
| 'kn2 ' | 0.0022 |
| 'kp　' | -0.1053 |
| 'kdp ' | 0.1197 |
| 'kdeg ' | 0.2162 |
| 'kdegs ' | -0.0108 |
| 'ki　' | 0.3173 |
| 'kis ' | -0.0439 |
| 'krec ' | -0.2369 |
| 'krecs ' | -0.0203 |
| 'kbind ' | 0.0932 |
| 'kdb　' | -0.0382 |
| 'kpakt ' | 0.1088 |
| 'kdpakt' | -0.2936 |
| 'ksyn ' | 0.3183 |
| 'Akt　' | 0.0938 |
| 'pAkt0 ' | 0.2874 |
| 'sEGFR0' | 0.1032 |
| **sEGFR steady state** | **0.3954** |

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202 **Supplemental Information Table 6. Correlation of parameters with steady state**
203 **pAkt levels at EGF = 10 ng/ml. Related to Figure 4.**

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| Allowed ranges | Experimental | | In silico | |
|---|---|---|---|---|
| | | | | |
| pAkt | lowe bound | upper bound | lower bound | upper bound |
| 0 ng/ml | 1.0123 | 139 | 1.0123 | 139 |
| 0.1 ng/ml 5min | 6.2849 | 276.6 | 6.2849 | 276.6 |
| 0.1 ng/ml 15min | 7.1279 | 321.6 | 7.1279 | 321.6 |
| 0.1 ng/ml 30min | 2.0056 | 265.6 | 2.0056 | 265.6 |
| 0.1 ng/ml 45min | 3.6327 | 263 | 3.6327 | 263 |
| 0.31 ng/ml 5min | 11.9039 | 441.8 | 11.9039 | 441.8 |
| 0.31 ng/ml 15min | 15.9734 | 540.6 | 15.9734 | 540.6 |
| 0.31 ng/ml 30min | 6.1803 | 396.9 | 6.1803 | 396.9 |
| 0.31 ng/ml 45min | 6.1442 | 281.4 | 6.1442 | 281.4 |
| 3.1 ng/ml 5min | 14.2768 | 896 | 14.2768 | 896 |
| 3.1 ng/ml 15min | 20.48 | 857.6 | 20.48 | 857.6 |
| 3.1 ng/ml 30min | 37.5317 | 563 | 37.5317 | 563 |
| 3.1 ng/ml 45min | 4.3151 | 344.4 | 4.3151 | 344.4 |
| 10 ng/ml 5min | 52.7802 | 1123 | 52.7802 | 1123 |
| 10 ng/ml 15min | 19.1342 | 869.4 | 19.1342 | 869.4 |
| 10 ng/ml 30min | 12.4531 | 627.2 | 12.4531 | 627.2 |
| 10 ng/ml 45min | 7.0271 | 397.3 | 7.0271 | 397.3 |
| 100 ng/ml 5min | 26.5866 | 1235.6 | 26.5866 | 1235.6 |
| 100 ng/ml 15min | 13.3069 | 921.2 | 13.3069 | 921.2 |
| 100 ng/ml 30min | 18.592 | 620.9 | 18.592 | 620.9 |
| 100 ng/ml 45min | 12.5386 | 462.9 | 12.5386 | 462.9 |
| | | | | |
| sEGFR | lower bound | upper bound | lower bound | upper bound |
| 0 ng/ml | 102.7394 | 664.5042 | 102.7394 | 664.5042 |
| 1 ng/ml | 23.4873 | 198.7291 | 23.4873 | 198.7291 |
| 100 ng/ml | 0.0819 | 106.7056 | 0.0819 | 106.7056 |

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212 **Supplemental Information Table 7. Allowed upper and lower bounds for pAkt and**
213 **sEGFR when inferring parameter distributions from data. Related to Figure 3 and**
214 **4.**