Supplementary Material
“Measuring fast gene dynamics in single cells with timelapse luminescence microscopy”

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Figure S1: Emission spectrum of different luciferases. We measured the ex vivo emission spectrum of designed beetle luciferases using a F900 fluorescent spectrometer (Edinburgh Instruments, UK). Yeast strains AMV104, AMV68, AMV69, AMV16, and AMV45 were pre-grown in SCD-Met at 30°C, washed, lysed and re-suspended with substrate before measurement. The pH of yeast extract lies between ~6-7. The ex vivo emission spectrum was normalized by peak intensity for each strain. Peak wavelengths are indicated in the legend. Published in vitro peak emission wavelengths of NLuc, GrLuc, YeLuc, FLuc, RdLuc are 460 nm, 538 nm, 560 nm, 562 nm, and 623 nm, respectively (Viviani et al., 1999a; 1999b; Hall et al., 2012). Our click beetle luciferases, GrLuc and RdLuc, were consistent with in vitro peak emission. However, peak emission of NLuc was blue-shifted ~10 nm, and the firefly luciferases (FLuc, YeLuc) were red-shifted ~50 nm. The spectrum of firefly luciferases was broader than the click beetle or marine luciferases. These results are consistent with previous reports, which show that click beetle and railroad worm luciferases are pH-insensitive, where as firefly luciferases are red shifted and have broader emission spectra in more acidic medium (Viviani et al., 2002). Our data suggest that NLuc, GrLuc, and RdLuc would be the best combination with minimal spectral overlap for multicolor luminescence.
Figure S2: Induction and repression dynamics of MET17 promoter in single cells for different luciferases. Our strains AMV104, AMV68, AMV69, AMV152-03, AMV45, and AMV151-08 were grown at 30°C in a CellAsic microfluidic device, which was mounted on DeltaVision microscope stage within an incubation chamber. Each luminescence image is a sum projection of 5 z-stacks separated by 0.4 µm, with 10 s exposures each stack for sub-minute total. Strains in (A,C,E,G,I,K) were pre-grown in SCD–Met and switched to repressive conditions, SCD+Met, at 360 or 390 minutes (vertical lines). Strains in (B,D,F,H,J,L) were pre-grown in SCD+Met and switched to inducing conditions, SCD–Met, at 360 or 390 minutes (vertical lines). Image segmentation of single cells was done with CellStat. Rapid increases in signal indicate new daughter cells identified and tracked by the segmentation program. Best fit of a mathematical model of gene repression with delay to average luminescence signal (dark colors) is shown in black; see Materials & Methods and Table S1 for details. The luminescence background (dashed, horizontal trace) is set by EMCCD camera noise. (A–B) MET17pr–NLS–GrLuc–ssrA, (C–D) MET17pr–NLS–YeLuc–ssrA, (E–F) MET17pr–NLS–RdLuc–ssrA, (G–H) MET17pr–CBG99, (I–J) MET17pr–FLuc, (K–L) MET17pr–CBR.
Figure S3: Induction and repression dynamics of LEU1 promoter in single cells for different luciferases. Our strains AMV141, AMV150, AMV154, and AMV153 were grown at 30°C on a CellAsic microfluidic device, which was mounted on DeltaVision microscope stage within an incubation chamber. Each luminescence image is a sum projection of 5 z-stacks separated by 0.4 µm, with 10 s exposures each stack for sub-minute total. Strains in (A,C,E,G) were pre-grown in SCD–Leu and switched to repressive conditions, SCD+Leu, at 360 or 390 minutes (vertical lines). Strains in (B,D,F,H) were pre-grown in SCD+Leu and switched to inducing conditions, SCD–Leu, at 360 or 390 minutes (vertical lines). Image segmentation of single cells was done with CellStat. Rapid increases in signal at beginning of time-course are the new daughter cells identified and tracked by the segmentation program. Best fit of a simple mathematical model of gene induction and repression with delay to average luminescence signal (dark colors) is shown in black; see Materials & Methods and Table S1 for details. The luminescence background (dashed, horizontal trace) is set by EMCCD camera noise. (A–B) LEU1pr–NLS–GrLuc–ssrA, (C–D) LEU1pr–FLuc, (E–F) LEU1pr–CBG99, (G–H) LEU1pr–CBR
Figure S4: Induction and repression dynamics of ADE17 and LYS9 promoter in single cells for different luciferases. Our strains AMV138, AMV148, AMV144, and AMV149 were grown at 30°C on a CellAsic microfluidic device, which was mounted on DeltaVision microscope stage within an incubation chamber. Each luminescence image is a sum projection of 5 z–stacks separated by 0.4 µm, with 10 s exposures each stack for sub–minute total. Strains in (A,C,E,G) were pre–grown in SCD–Ade or SCD–Lys and switched to repressive conditions, SCD+Ade or SCD+Lys, at 300 minutes (vertical lines). Strains in (B,D,F,H) were pre–grown in SCD+Ade or SCD+Lys and switched to inducing conditions, SCD–Ade or SCD–Lys, at 300 minutes (vertical lines). Image segmentation of single cells was done with CellStat. Rapid increases in signal at beginning of time–course are the new daughter cells identified and tracked by the segmentation program. Best fit of a simple mathematical model of gene induction and repression with delay to average luminescence signal (dark colors) is shown in black; see Materials & Methods and Table S1 for details. The luminescence background (dashed, horizontal trace) is set by EMCCD camera noise. (A–B) ADE17pr–NLS–GrLuc–ssrA, (C–D) ADE17pr–FLuc, (E–F) LYS9pr–NLS–GrLuc–ssrA, (G–H) LYS9pr–FLuc.
**Figure S5:** Induction and repression dynamics of LEU1 promoter driving luciferase-fluorescent protein fusion in single cells. Our strain AMV166 was grown at 30°C on a CellAsic microfluidic device, which was mounted on DeltaVision microscope stage within an incubation chamber. Each luminescence image is a sum projection of 5 z–stacks separated by 0.4 µm, with 10 s exposures each stack for sub–minute total. In the middle of the z-stack, we acquired a 2 msec fluorescence image (green) followed by a 7 msec phase image for image segmentation. Strains in (A) were pre–grown in SCD–Leu and switched to repressive conditions, SCD+Leu, at 390 minutes (vertical line). Strains in (B) were pre–grown in SCD+Leu and switched to inducing conditions, SCD–Leu, at 390 minutes (vertical line). Image segmentation of single cells was done with CellStat. Rapid increases in signal at beginning of time–course are the new daughter cells identified and tracked by the segmentation program. Best fit of a simple mathematical model of gene induction and repression with delay to average luminescence or fluorescence signal (thick, colored lines) is shown in black; see Materials & Methods and Table S1 for details. The luminescence and fluorescence background (dashed, horizontal trace) is set by EMCCD camera noise and cell auto–fluorescence, respectively. (A, B) Repressed and induced LEU1pr-FLuc-yEVenus.
Figure S6: Induction and repression dynamics of MET17 promoter driving luciferase-fluorescent protein fusion both with and without PEST degron. Our strains AMV50 and AMV63 were grown at 30°C on a CellASIC microfluidic device, which was mounted on DeltaVision microscope stage within an incubation chamber. Each luminescence image is a sum projection of 5 z-stacks separated by 0.4 µm, with 10 s exposures each stack for sub-minute total. In the middle of the z-stack, we acquired a 2 msec fluorescence image (green) followed by a 7 msec phase image for image segmentation. Strains in (A, C) were pre-grown in SCD-Met and switched to repressive conditions, SCD+Met, at 240 minutes (vertical line). Strains in (B, D) were pre-grown in SCD+Met and switched to inducing conditions, SCD-Met, at 240 minutes (vertical line). Image segmentation of single cells was done with CellStat. Rapid increases in signal at beginning of time-course are the new daughter cells identified and tracked by the segmentation program. Best fit of a simple mathematical model of gene induction and repression with delay to average luminescence or fluorescence signal (thick, colored lines) is shown in black; see Materials & Methods and Table S1 for details. The luminescence and fluorescence background (dashed, horizontal trace) is set by EMCCD camera noise and cell auto-fluorescence, respectively. (A,B)
Repressed and induced \( MET17pr-FLuc-yEVenus \), (C,D) Repressed and induced \( MET17pr-FLuc-yEVenus-PEST \)

Figure S7: Comparison of FLuc and yEVenus-PEST dynamics. Single-cell timelapse luminescence and fluorescence microscopy of \( SIC1 \) or \( RNR1 \) promoter in yeast strains AMV137, AMV156 or AMV164, AMV165 with (A) \( SIC1pr-FLuc \), (B) \( SIC1pr-yEVenus-PEST \), (C) \( RNR1pr-FLuc \), (D) \( RNR1pr-yEVenus-PEST \). Filming and image segmentation was similar to details in Figure 5. The raw, noisy luminescence and fluorescence were smoothed with a Savitzky-Golay filter (with a span of eight data points and one polynomial degree) to reliably detect peak \( SIC1 \) or \( RNR1 \) expression. Rapid increase in signal from background is the new bud identified and tracked by the segmentation program. The vertical, dashed lines in individual traces correspond to budding, a visible cell cycle event. Statistical differences in timing between budding and peak time are reported in the Table beneath the figures.
Table S1: Induction and repression dynamics across promoters and luciferases. A summary of the best-fit parameters (along with 95% confidence interval, CI) for a simple mathematical model of gene expression fit to the mean luminescence data shown in Figures 4-5 and S2–S6. For FLuc-yEVENUS and FLuc-yEVENUS-PEST fusion construct, each row
corresponds to the best-fit parameters to either luminescence or fluorescence signal (indicated with underline and bold font). Cell doubling time ($\lambda$) was also inferred from timelapse data. The model and fitting procedure is described in Materials & Methods. Our results demonstrated an asymmetry between the kinetics of induction and repression of metabolite-repressed promoters, when averaged over luciferases. **LEU1, ADE17, LYS9** promoters exhibited a fast ON, slow OFF response with a longer delay ($\tau \sim$30-40 minutes) during metabolite repression and a shorter, variable delay ($\tau \sim$5-20 minutes) upon induction. (Fast ON, slow OFF). **MET17** was an outlier with opposite behavior; namely, a longer delay during induction ($\sim$30 minutes) and a shorter delay ($\sim$15 minutes) during repression. We do not have an explanation for this difference, but we note that methionine (unlike the other metabolites) plays an important role in cell growth, redox homeostasis, and metabolism (Petti et al., 2012). Such metabolic changes might affect luminescence because luciferases are sensitive to changes in pH, ATP, and redox.
**Table S2**: Statistical analysis of peak luminescence and/or fluorescence minus budding time distributions for SIC1 and RNR1 promoters. Strains that were compared in pair-wise fashion are listed in the top row and left column. We analyzed strain luminescence or fluorescence signal when FLuc or YFP (i.e. yEVenus) is underlined, respectively. Our null hypothesis is that distributions are identical. The probability (p-value) that this hypothesis is correct for each pair of distributions was calculated using a two-sample Student’s t-test with unequal variance. We used 1E-03 as a conservative, p-value threshold for significance (Johnson, 2013). Red fonts indicate distributions that are not identical, whereas black fonts are those distributions that are likely identical. We clustered strains in rows by their p-values. Boxes indicate triplet clusters where 2 out of 3 members have similar distributions (p > 0.001, black font). Dark grey, solid boxes are promoter-reporter pairs with identical promoters and reporters. Light grey, dashed boxes are promoter-reporter pairs where the promoters are different, but the reporter is identical. The light grey boxes exist because

|       | SIC1 pr-FLuc | SIC1 pr-YFP | RN1 pr-FLuc | RN1 pr-YFP | SIC1 pr-FLuc | SIC1 pr-YFP | RN1 pr-FLuc | RN1 pr-YFP | SIC1 pr-FLuc | SIC1 pr-YFP | RN1 pr-FLuc | RN1 pr-YFP |
|-------|--------------|-------------|-------------|------------|--------------|-------------|-------------|------------|--------------|-------------|-------------|------------|
| Source| Figure S7    | Figure S7   | Figure S7   | Figure S7  | Figure S7    | Figure S7   | Figure S7   | Figure S7  | Figure S7    | Figure S7   | Figure S7   | Figure S7 |
| 1E+00 | 2E-20        | 8E-05       | 3E-33       | 8E-02      | 3E-11        | 6E-02       | 2E-18       | 6E-08      | 8E-21        | 5E-02       | 2E-22       |
| 8E-02 | 2E-08        | 3E-01       | 4E-13       | 1E+00      | 3E-06        | 2E-03       | 2E-08       | 8E-03      | 5E-11        | 8E-01       | 5E-12       |
| 6E-02 | 4E-23        | 6E-08       | 3E-33       | 2E-03      | 1E-13        | 1E+00       | 4E-21       | 5E-11      | 3E-23        | 3E-04       | 9E-25       |
| 2E-20 | 1E+00        | 6E-12       | 2E-05       | 2E-08      | 5E-01        | 4E-23       | 9E-01       | 5E-06      | 2E-02        | 2E-14       | 2E-03       |
| 3E-11 | 5E-01        | 2E-06       | 1E-04       | 3E-06      | 1E+00        | 4E-23       | 5E-11       | 2E-03      | 1E-02        | 2E-08       | 2E-03       |
| 2E-18 | 9E-01        | 6E-11       | 1E-04       | 2E-08      | 4E-01        | 4E-21       | 1E+00       | 7E-06      | 4E-02        | 2E-13       | 6E-03       |
| 8E-05 | 6E-12        | 1E+00       | 7E-25       | 3E-01      | 2E-06        | 6E-08       | 6E-11       | 3E-02      | 3E-14        | 6E-02       | 7E-16       |
| 6E-08 | 5E-06        | 3E-02       | 4E-15       | 8E-03      | 2E-03        | 5E-11       | 7E-06       | 1E+00      | 3E-09        | 2E-04       | 9E-11       |
| 5E-02 | 2E-14        | 6E-02       | 2E-24       | 8E-01      | 2E-08        | 3E-04       | 2E-13       | 2E-04      | 2E-16        | 1E+00       | 5E-18       |
| 3E-33 | 2E-05        | 7E-25       | 1E+00       | 4E-13      | 1E-04        | 3E-33       | 1E-04       | 4E-15      | 2E-01        | 2E-24       | 6E-01       |
| 8E-21 | 2E-02        | 3E-14       | 2E-01       | 5E-11      | 1E-02        | 3E-23       | 4E-02       | 3E-09      | 1E+00        | 2E-16       | 5E-01       |
| 2E-22 | 2E-03        | 7E-16       | 6E-01       | 5E-12      | 2E-03        | 9E-25       | 6E-03       | 9E-11      | 5E-01        | 5E-18       | 1E+00       |
**SIC1** peaks ~5 minutes before **RNRI**, which is close to the variability of gene expression and temporal resolution of our timelapse microscopy (i.e. snapshot every 4 minutes).

| Cell type, organism | Cell size (µm) | Camera | Objective | Exposure (secs) | Luciferase | Substrate | Ref. |
|---------------------|----------------|--------|-----------|-----------------|------------|-----------|------|
| HeLa cells, *H. sapiens* | 15 | I-CCD | 40x | 1800 | Firefly(FLuc) | D-Luciferin | (White et al., 1995) |
| GH3 cells, *R. novegicus* | 10 | I-CCD | 10x | 1800 | Firefly(FLuc) | D-Luciferin | (Stirland et al., 2003) |
| Cyanobacteria, *S. elongatus* | 3 | btCCD | 100x | 1800 | Bacteria(LuxAB) | N-Decanal | (Mihalcescu et al., 2004) |
| Rat–1 fibroblast, *M. musculus* | 10 | btCCD | 4x | 1800 | Firefly(FLuc) | D-Luciferin | (Welsh et al., 2004) |
| Cells, *D. rerio* | 10 | I-CCD | Unknown | 1800 | Firefly(FLuc) | D-Luciferin | (Carr and Whitmore, 2005) |
| NIH3T3 cells, *M. musculus* | 15 | btEM–CCD | 20x | 1740 | Beetle(ELuc) | D-Luciferin | (Kwon et al., 2010) |
| Rat–1 fibroblast, *M. musculus* | 10 | CCD | 20x | 1740 | Marine(GLuc) | Coelentrenzine | (Yeom et al., 2010) |
| NIH3T3 cells, *M. musculus* | 15 | CCD | 10x | 1500 | Firefly(FLuc) | D-Luciferin | (Sato et al., 2006) |
| NIH3T3 cells, *M. musculus* | 15 | CCD | 10x | 1500 | Firefly(FLuc) | D-Luciferin | (Ukai et al., 2007) |
| SCN neuron, *M. musculus* | 9 | cryo-CCD | Unknown | 1200 | Firefly(FLuc) | D-Luciferin | (Yamaguchi et al., 2003) |
| CHO–T cells, *C. griseus* | 13 | I-CCD | 10x | 900 | Firefly(FLuc) | D-Luciferin | (Krug et al., 1995) |
| Pituitary cells, *R. novegicus* | 7 | I-CCD | 40x | 900 | Firefly(FLuc) | D-Luciferin | (Castano et al., 1996) |
| SR1 plant, *N. tabacum* | 1000 | I-CCD | Unknown | 600 | Firefly(FLuc) | D-Luciferin | (Millar et al., 1992) |
| Astrocytes, *R. novegicus* | 15 | btEM–CCD | 4x | 540 | Beetle(ELuc) | D-Luciferin | (Nakajima et al., 2010) |
| HeLa cells, *H. sapiens* | 15 | I-CCD | 10x | 300 | Firefly(FLuc) | D-Luciferin | (White et al., 1999) |
| Mouse neuron, *M. musculus* | 20 | cryo-CCD | 40x | 300 | Firefly(FLuc) | D-Luciferin | (Asai et al., 2008) |
| NIH3T3 cells, *M. musculus* | 15 | btEM–CCD | 20x | 300 | Firefly(FLuc) | D-Luciferin | (Suter et al., 2011) |
| NIH3T3 cells, *M. musculus* | 15 | btEM–CCD | 40x | 180 | Beetle(ELuc) | D-Luciferin | (Nakajima et al., 2010) |
| CHO–T cells, *C. griseus* | 13 | I-CCD | 10x | 60 | Marine(Aequorin) | Coelentrenzine/Ca ** | (Rutter et al., 1995) |
| Yeast, *S. cerevisiae* | 3.5 | btEM–CCD | 60x | 50 | Firefly/Beetle | D-Luciferin | (Hoshino et al., 2007) |
| NIH3T3 cells, *M. musculus* | 15 | btEM–CCD | 20x | 10 | EYFP–RLuc | Coelentrenzine | (Saito et al., 2010) |
| HeLa cells, *H. sapiens* | 15 | btEM–CCD | 40x | 1 | Venus–RLuc | Coelentrenzine | (Saito et al., 2012) |
| HeLa cells, *H. sapiens* | 15 | btEM–CCD | 60x | 1 | EYFP–RLuc | Coelentrenzine | (Saito et al., 2012) |

**Table S3**: Timelapse luminescence microscopy across different organisms. We ranked live-cell timelapse luminescence microscopy results from longest to shortest exposure time (in seconds). Cell size is cell diameter estimated from the literature. The CCD cameras are either intensified CCD (I-CCD), btCCD (back-thinned CCD), cryo-CCD (cryogenically cooled CCD), btEM-CCD (back-thinned, electron-multiplying CCD). For comparison, our beetle luciferase results in yeast are highlighted in red.
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