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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

Fig 1a-c. Sample size was not pre-determined. See Table 1 below.

Fig 1d. Y-axis. Sample size was not pre-determined. The mean was derived from 75 +/-20 cells. The error is the SEM and is on average 20%. X-axis. Net protein expression was derived from two replicates for each FP. This is enough to distinguish 20% changes in net protein expression. Compared to the range of relative fluorescence signal covered by our FP library (see range of X- and Y-axis in Fig 1d), data errors in the X- and Y-axis are almost 10 times lower and, as quantified by the r-squared, allows us to clearly distinguish the effect of maturation time between Fig. 1d and Fig. 1e.

Fig 1e. Y-axis. Same as Fig 1d. Y-axis. X-axis. Sample size was not predetermined. Quantum yield and molar extinction were derived from 3 protein extractions. This is enough to distinguish 15% changes in in vitro brightness. Net protein expression was derived from two replicates for each fluorescent protein. This is enough to distinguish 20% changes in net protein expression. The t50 maturation time error is below 10%. Propagating the previous errors results in about a 20% error in the estimated relative fluorescence. This error is almost 10 times lower than the range of relative fluorescence signal covered by our FP library in Fig 1d-e and, as quantified by the r-squared, allows us to clearly distinguish the effect of maturation time between Fig. 1d and Fig. 1e.

Fig. 2b, Fig. 2e, S Fig. 15 and S Fig. 16. Sample size was not pre-determined. mGFPmut2, ncell=2489, mEGFP, ncell= 2581, mTurquoise2 ncells=1460 and SCFP3A ncells=1711. Fig.2b. Sample size is large enough that fluctuations in the tail of the distribution are only seen after the 99th percentile. Fig. 2e and S Fig. 16b. The sampling of the autocorrelation functions diminishes as the lag time increases. However, the sampling was high enough at lag time 80min in Fig. 2e (n=12000) and at lag time 150min (n=7500) in Fig. 16b to ignore fluctuations. S Fig. 15 and S Fig. 16c. Sample size was enough to have a robust estimation of the dynamic range, i.e. the dynamic range ratio (fast FP)/(slow FP) is similar using the 1st and the 99th percentile, or using the 2nd and the 98th percentile.

Table 1. Sample size was not pre-determined. Maturation curves were calculated from 75+/-20 cells. At 25% and at 100% fluorescence, the experimental fluctuation of maturation curves (local CV value) is, for the great majority, well below 3% and 1%, respectively. Because the trend of the maturation curve (obtained by a smoothing filter) is robust to experimental fluctuations, we derived reliable t50 and t90 values from all curves and reported 95% confidence intervals.
To see fine details of the maturation kinetics at, e.g. 90% fluorescence, error needs to be lower than 1:10. As mentioned above, our error around 100% fluorescence saturation is below 1:100. Thus, for almost all curves, fluctuations around the mean trend are very low and allow to see the fine details of the maturation kinetics. Exceptions are, at 100% fluorescence, DsRedEx 32°C (4.9%), TagRFP 32°C (3.1%), TagRFP-T 32°C (4.7%).

2. Data exclusions

Describe any data exclusions.

Fig. 1 and Table 1. A few cells would anomalously lose fluorescence after ~2hrs of chloramphenicol treatment due, presumably, to cell-wall damage. We plotted the single-cell fluorescence vs time of all tracked cells to visually detect and eliminate those cells from the analysis. Cells were eliminated because including them would have created artificial photobleaching and thus would have altered maturation kinetics measurements.

Fig. 2 and S Fig. 16. An experiment in the single-cell chemostat is recorded using many fields of view (FOV). Some FOVs are not analyzable because (i) local fluorescence background is too high due to bad buffer flow, (ii) or cells do not grow along the linear tracks and create biofilms instead of linear colonies or (iii) the microscope software would fail to track the FOV. We only included in the analysis, linear colonies from FOV without these problems.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Fig. 1 and Table 1. Immature FP fraction curves were initially obtained for several FPs (mEGFP, mGFPmut2, mGFPmut3, sfGFP, SCFP3A, mVenME) with at least 3 independent replicates. Once the results from these initial FPs were reproducible, new FPs were measured together with a previously characterized FP as a control. If the control displayed an anomalous maturation curve, the experiment was not further analyzed.

Fig. 2 and S Fig. 16. We performed a single experiment with FPs of different colors: two greens (mGFPmut2 & mEGFP) and two blues (SCFP3A & mTurquoise2) to support the robustness of the observed effect.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

It does not apply to our work because we do not have different experimental groups, e.g. a treatment and a control group.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding is not relevant to our study because we knew the identity of every measured FP.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☒	☐ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☒	☐ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this

We used custom software to obtain, from time lapses, kymographs of linear

study.

colonies and to track and quantify the fluorescence of cells. The software is available upon request. All software was written in Matlab R2013a.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

The study did not involve research animals.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Three replicate cultures for every avFP were grown overnight in M9 rich media at 37°C. Next day, a first set of replicates was diluted 1000X in fresh M9 rich media and incubated at 37°C. After 20 min, the same procedure was followed for the second set of replicates and, finally, after an additional 20 more minutes the third set was also diluted and incubated. The delay between replicates was set to minimize maturation time artifacts in the in vivo brightness determination using flow cytometry. Typically, a single set of replicates would take ~6min to be quantified. After 2hrs 40min from the first dilution, a second 500x dilution was performed for every set of replicates following the same time delay. After 2hrs, the replicates were growing exponentially (OD600 = 0.05-0.1). An aliquot of the first set was transferred to a 96-well plate pre warmed to 37°C and stored in a styrofoam box. Immediately, samples were measured in a BD LSR Fortessa. The same was done for the second and the third bio-replicate sets.
6. Identify the instrument used for data collection.	Samples were measured in a BD LSR Fortessa. The excitation/emission configuration was CFP Ex 440 (laser), Em 470/20; GFP Ex 488 (laser), Em 520/35; and YFP Ex 488 (laser), Em 542/27.
7. Describe the software used to collect and analyze the flow cytometry data.	Flowing Software 2.5.1
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Greater than 95%
9. Describe the gating strategy used.	For green FPs, cell-like objects were separated from a clear debris fraction by using the side and forward scattering. Then, cell objects were identified by gating only events with green fluorescence (FITC channel). The same was done for yellow FPs and cyan FPs. For the latter, the cyan channel was used in the second gaiting. We have exemplified the gating strategy in Supplementary Figure 11.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒