Supplementary statistical analysis for

Versatile phenotype-activated cell sorting

General comments

For each experiment, we have noted down sample sizes (n), and the types of statistical analysis, and the result of the analysis. For all statistical analysis, significance level of 0.05 was used. Exact *p* value is given except when *p* value is < 0.0001.

For sample size greater than 30, normality test was not conducted, and parametric analysis was used. This because violation of normality has a small effect on parametric analysis when the sample size is large (Ref: 64).

To determine the statistical difference between trends, we have calculated areas under the curves (AUCs) and conducted statistical analysis using the AUCs.

Figure 2B

Bacteria

Sample size

	Column A: Neighboring	Column B: Target
n	7 cells	7 cells

To analyze statistical difference between the photoactivation trends, areas under the curves (AUCs) of photoactivation ratio (RFP/RFP_0) vs. photoactivation duration were determined for individual cells. Logged AUC values were used for the statistical analysis.

Shapiro-Wilk normality test

Shapiro-Wilk test	Neighboring	Target
W	0.8215	0.7734
P value	0.0663	0.0221
Passed normality test (alpha=0.05)?	Yes	No
P value summary	ns	*

➔ Normality test was not passed.

Mann Whitney test

Mann Whitney test	
P value	0.0012
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	29 , 76
Mann-Whitney U	1
Difference between medians	
Median of column A	2.836, n=7
Median of column B	3.900, n=7
Difference: Actual	1.063
Difference: Hodges-Lehmann	1.035

➔ The logged AUCs values are significantly different.

Yeast

Sample size

	Column A: Neighboring	Column B: Target
n	18 cells	18 cells

To analyze the statistical difference between the photoactivation trends, areas under the curves (AUCs) of photoactivation ratio (RFP/RFP_0) vs. time were determined for individual cells. Logged AUC values were used for the statistical analysis.

Shapiro-Wilk normality test

Shapiro-Wilk test	Neighboring	Target
W	0.9516	0.9600
P value	0.4502	0.6016
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

➔ Normality test was passed.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	1.141, 17, 17
P value	0.7893
P value summary	ns
Significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Unpaired t test

Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=16.40, df=34
How big is the difference?	
Mean of column A	2.663
Mean of column B	4.496
Difference between means (B - A) ± SEM	1.832 ± 0.1118
95% confidence interval	1.605 to 2.059
R squared (eta squared)	0.8877

➔ The logged AUCs values are significantly different.

Human cell

Sample size

	Neighboring	Target
n	5 cells	5 cells

To analyze the statistical difference between the photoactivation trends, areas under the curves (AUCs) of photoactivation ratio (RFP/RFP_0) vs. time were determined for individual cells. Logged AUC values were used for the statistical analysis.

Shapiro-Wilk normality test

Shapiro-Wilk test		
W	0.9022	0.8778
P value	0.4222	0.2997
Passed normality test (alpha=0.05)?	Yes	Yes

P value summary	ns	ns

➔ Both neighboring and target cell groups passed the normality test.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	16.85, 4, 4
P value	0.0181
P value summary	*
Significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Unpaired t test with Welch's correction

Unpaired t test with Welch's correction	
P value	0.0105
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=4.227, df=4.473

➔ The logged AUCs values are significantly different.

Figure 3

Figure 3B: Human cell

Sample

	Column A: Neighboring	Column B: Target
n	12	12

To analyze the statistical difference between the photoactivation trends, areas under the curves (AUCs) of photoactivation ratio (RFP/RFP_0) vs. time were determined for individual cells. Logged AUC values were used for the statistical analysis.

Shapiro-Wilk normality test

Shapiro-Wilk test	Neighboring	Target
W	0.8268	0.9114
P value	0.0192	0.2222
Passed normality test (alpha=0.05)?	No	Yes
P value summary	*	ns

➔ Neighboring cell failed the normality test.

Mann Whitney test

Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	78 , 222
Mann-Whitney U	0

➔ The logged AUCs values are significantly different.

Figure 3F: Human intestinal enteroids

Sample size

	Column A: Neighboring	Column B: Target
n	11 enteroids	11 enteroids

To analyze the statistical difference between the photoactivation trends, areas under the curves (AUCs) of photoactivation ratio (RFP/RFP_0) vs. time were determined for individual enteroids. Logged AUC values were used for the statistical analysis.

Shapiro-Wilk normality test

Shapiro-Wilk test	Neighboring	Target
W	0.9376	0.8259
P value	0.4932	0.0206
Passed normality test (alpha=0.05)?	Yes	No
P value summary	ns	*

➔ Normality test was not passed.

Mann Whitney test

Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	66 , 187
Mann-Whitney U	0
Difference between medians	
Median of column A	145.3, n=11
Median of column B	1496, n=11
Difference: Actual	1351
Difference: Hodges-Lehmann	1322

➔ The logged AUCs values are significantly different.

Figure 4H

Sample size

	Column A: Before photoactivation	Column B: After photoactivation
Neighboring	45 cells	45 cells

Logged fluorescence values were used for statistical analysis.

Ratio paired t test

Ratio paired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes

One- or two-tailed P value?	Two-tailed
t, df	t=79.46, df=44
Number of pairs	45
How big is the difference?	
Geometric mean of ratios (B / A)	2.013
SD of log(ratios)	0.02565
SEM of log(ratios)	0.003824
95% confidence interval	1.978 to 2.049
R squared (partial eta squared)	0.9931
How effective was the pairing?	
Correlation coefficient (r)	0.8938
P value (one tailed)	<0.0001
P value summary	****
Was the pairing significantly effective?	Yes

➔ The logged fluorescence values are significantly different.

Figure 5

Figure 5A: Photobleaching half-life comparison in yeast

Sample size (cells)

	mVenus			mCitrine		
	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
Trial 1	1143	694	1341	864	776	1614
Trial 2	1988	2817	2872	1010	2692	659
	Ypet			mGold		
	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
Trial 1	420	700	742	2363	484	603
Trial 2	1939	2319	4613	1493	3225	2960

For each replicate, the photobleaching half-lives of individual cells were determined and the mean photobleaching half-life was calculated (of the replicate). These mean photobleaching half-life values were used for statistical analysis. n = 6 biological replicates.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	3.859 (3, 20)
P value	0.0250
P value summary	*
Are SDs significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Welch's ANOVA test

Welch's ANOVA test	
W (DFn, DFd)	134.4 (3.000, 10.76)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

➔ The photobleaching half-life values are significantly different.

Dunnett's T3 multiple comparisons test

Dunnett's T3 multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
mGold vs. mVenus	71.65	59.23 to 84.07	Yes	****	<0.0001			
mCitrine vs. mGold	-68.53	-81.01 to -56.04	Yes	****	<0.0001			
Ypet vs. mGold	-65.46	-78.06 to -52.87	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
mGold vs. mVenus	95.79	24.15	71.65	3.477	6	6	20.60	7.206
mCitrine vs. mGold	27.27	95.79	-68.53	3.496	6	6	19.60	7.319
Ypet vs. mGold	30.33	95.79	-65.46	3.337	6	6	19.61	6.330

➔ The photobleaching half-life values of mGold and mVenus are significantly different.

Figure 5A: Brightness comparison in yeast

Sample size (cells)

Same as above

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	0.6983 (3, 20)
P value	0.5640
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Ordinary one-way ANOVA test

ANOVA summary	
F	21.51
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

➔ The brightness values are significantly different.

Bonferroni's multiple comparisons test

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
mGold vs. mVenus	0.06078	-0.002570 to 0.1241	No	ns	0.0591			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
mGold vs. mVenus	0.5070	0.4463	0.06078	0.03037	6	6	2.001	20

➔ The brightness values of mGold and mVenus are not significantly different.

Figure 5A: Photobleaching half-life comparison in human cells

Sample size (cells)

	mVenus			mCitrine		
	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
Trial 1	183	206	152	215	320	170
Trial 2	165	161	140	191	159	182
	Ypet			mGold		

	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
Trial 1	175	235	325	219	197	208
Trial 2	234	215	127	216	267	164

For each replicate, the photobleaching half-lives of individual cells were determined and the mean photobleaching half-life was calculated (of the replicate). These mean photobleaching half-life values were used for statistical analysis. n = 6 biological replicates.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	7.235 (3, 20)
P value	0.0018
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Welch's ANOVA test

Welch's ANOVA test	
W (DFn, DFd)	3443 (3.000, 10.45)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

➔ The photobleaching half-life values are significantly different.

Dunnett's T3 multiple comparisons test

Dunnett's T3 multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
mGold vs. mVenus	168.4	163.0 to 173.9	Yes	****	<0.0001			
Ypet vs. mGold	-160.8	-167.9 to -153.6	Yes	****	<0.0001			
mCitrine vs. mGold	-186.1	-192.8 to -179.4	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
mGold vs. mVenus	218.9	50.41	168.4	1.830	6	6	92.07	6.338
Ypet vs. mGold	58.09	218.9	-160.8	1.749	6	6	91.90	5.387
mCitrine vs. mGold	32.74	218.9	-186.1	1.773	6	6	105.0	5.670

➔ The photobleaching half-life values of mGold and mVenus are significantly different.

Figure 5A: Brightness comparison in human cells

Sample size (cells)

Same as above.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	1.903 (3, 20)
P value	0.1617
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Ordinary one-way ANOVA test

ANOVA summary	
F	21.51
P value	<0.0001

P value summary	****
Significant diff. among means (P < 0.05)?	Yes

➔ The brightness values are significantly different.

Bonferroni's multiple comparisons test

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
mGold vs. mVenus	-0.1302	-0.4299 to 0.1695	No	ns	0.3757			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
mGold vs. mVenus	2.540	2.671	-0.1302	0.1437	6	6	0.9060	20

➔ The brightness values of mGold and mVenus are not significantly different.

Figure 5B: Photobleaching half-life vs. irradiance in yeast

Sample size (cells)

	Column A: mVenus			Column B: mGold		
Irradiance (mW/mm^2)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
6.01	1154	957	1265	727	1989	2193
10.9	1260	989	1265	662	2064	2196
14.6	1169	919	1292	758	2030	2087
17.6	1300	980	1312	860	2063	2199
20.1	1203	1024	1421	828	2002	639

For each replicate, the photobleaching half-lives of individual cells were determined and the mean photobleaching half-live (of the replicate) was calculated. These mean photobleaching half-life values were used to determine the AUCs of irradiance vs. photobleaching half-life curves. Statistical analysis was conducted on the AUCs; n = 3 biological replicates.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	43.47, 2, 2
P value	0.0450
P value summary	*
Significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Unpaired t test with Welch's correction

Unpaired t test with Welch's correction	
P value	0.0126
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=8.238, df=2.092
How big is the difference?	
Mean of column A	451.1
Mean of column B	2165

Difference between means (B - A) ± SEM	1714 ± 208.1
95% confidence interval	855.4 to 2572
R squared (eta squared)	0.9701

➔ The AUCs values are significantly different.

Multiple t-test with Holm-Sidak method

For each irradiance, t-test was conducted to analyze the difference between photobleaching half-life values of mVenus and mGold.

Irradiance (mW/mm^2)	Significant?	P value	Mean of mVenus	Mean of mGold	Difference	SE of difference	t ratio	df	Adjusted P Value
6.01	Yes	0.0098	62.16	233.3	-171.1	36.99	4.626	4.000	0.0098
10.9	Yes	0.0039	32.74	181.5	-148.8	24.76	6.009	4.000	0.0077
14.6	Yes	<0.0001	25.17	131.2	-106.0	6.239	16.99	4.000	0.0004
17.6	Yes	0.0008	19.80	96.82	-77.02	8.336	9.239	4.000	0.0023
20.1	Yes	0.0002	16.26	89.89	-73.63	5.800	12.69	4.000	0.0009

➔ The photobleaching half-life values at each irradiance are significantly different.

Figure 5B: Photobleaching half-life vs. irradiance in human cells

Sample size (cells)

	Column A: mVenus			Column B: mGold		
Irradiance (mW/mm^2)	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
6.01	196	184	313	330	267	242
10.9	199	289	273	351	331	260
14.6	n/a	307	326	341	272	117
17.6	179	298	321	262	257	151
20.1	185	288	252	270	215	252

For each replicate, the photobleaching half-lives of individual cells were determined and the mean photobleaching half-live (of the replicate) was calculated. These mean photobleaching half-life values were used to determine the AUCs of irradiance vs. photobleaching half-life curves. Statistical analysis was conducted on the AUCs; n = 3 biological replicates.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	8.274, 2, 2
P value	0.2156
P value summary	ns
Significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Unpaired t test

Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=40.76, df=4
How big is the difference?	
Mean of column A	1296

Mean of column B	5046
Difference between means (B - A) ± SEM	3750 ± 92.00
95% confidence interval	3495 to 4005
R squared (eta squared)	0.9976
Unpaired t test	

➔ The AUCs values are significantly different.

Multiple t-test with Holm-Sidak method

For each irradiance, t-test was conducted to analyze the difference between photobleaching half-life values of mVenus and mGold.

Irradiance (mW/mm^2)	Significant ?	P value	Mean of mVenus	Mean of mGold	Difference	SE of difference	t ratio	df	Adjusted P Value
6.01	Yes	<0.0001	185.2	513.3	-328.1	15.80	20.76	4.000	<0.0001
10.9	Yes	<0.0001	93.77	430.5	-336.8	3.729	90.32	4.000	<0.0001
14.6	Yes	<0.0001	69.40	301.6	-232.2	13.51	17.18	4.000	0.0001
17.6	Yes	<0.0001	55.16	245.0	-189.9	3.346	56.75	3.000	<0.0001
20.1	Yes	<0.0001	46.74	211.4	-164.7	9.375	17.57	4.000	0.0001

➔ The photobleaching half-life values at each irradiance are significantly different.

Figure S1

Figure S1A

Sample size (cells)

	PAmCherry1 Plasmid	PATagRFP Plasmid	PAmKate Plasmid	PAmCherry1 Genome
n	8848	6465	7271	6626

Areas under the curves (AUCs) were determined for individual photoactivation ratio curve of all cells analyzed (n = ~6000 to 9000). Logged AUC values were used for statistical analysis.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	1348 (3, 29206)
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Welch's ANOVA test

Welch's ANOVA test	
W (DFn, DFd)	17440 (3.000, 16105)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

➔ The logged AUCs values are significantly different.

Games-Howell's multiple comparisons test

Games-Howell's multiple comparisons test	Mean Diff.	95.00 % CI of diff.	Significant?	*Summary*	Adjusted P Value			

PAmCherry plasmid vs. PATagRFP plasmid	0.4825	0.4651 to 0.5000	Yes	****	<0.0001			
PAmCherry plasmid vs. PAmKate plasmid	1.343	1.327 to 1.360	Yes	****	<0.0001			
PAmCherry plasmid vs. PAmCherry genome	0.9879	0.9713 to 1.005	Yes	****	<0.0001			
PATagRFP plasmid vs. PAmKate plasmid	0.8607	0.8463 to 0.8750	Yes	****	<0.0001			
PATagRFP plasmid vs. PAmCherry genome	0.5054	0.4910 to 0.5198	Yes	****	<0.0001			
PAmKate plasmid vs. PAmCherry genome	-0.3553	-0.3687 to -0.3419	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
PAmCherry plasmid vs. PATagRFP plasmid	5.075	4.592	0.4825	0.006788	8848	6465	71.09	15216
PAmCherry plasmid vs. PAmKate plasmid	5.075	3.732	1.343	0.006480	8848	7271	207.3	15118
PAmCherry plasmid vs. PAmCherry genome	5.075	4.087	0.9879	0.006481	8848	6626	152.4	14809
PATagRFP plasmid vs. PAmKate plasmid	4.592	3.732	0.8607	0.005588	6465	7271	154.0	13256
PATagRFP plasmid vs. PAmCherry genome	4.592	4.087	0.5054	0.005590	6465	6626	90.41	12829
PAmKate plasmid vs. PAmCherry genome	3.732	4.087	-0.3553	0.005211	7271	6626	68.17	13864

Figure S1B

Sample size

	PAmCherry1 Plasmid	PATagRFP Plasmid	PAmKate Plasmid	PAmCherry1 Genome
n	8848 cells	6465 cells	7271 cells	6626 cells

Photoactivation t-half values were determined for individual cells analyzed (n = ~6000 to 9000). Logged t-half values were used for statistical analysis.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	284.2 (3, 29204)
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Welch's ANOVA test

Welch's ANOVA test	
W (DFn, DFd)	53642 (3.000, 14797)
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes

➔ The logged t-half values are significantly different.

Games-Howell's multiple comparisons test

Games-Howell's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
PAmCherry1 plasmid vs. PATagRFP plasmid	-0.4189	-0.4225 to -0.4153	Yes	****	<0.0001			
PAmCherry1 plasmid vs. PAmKate plasmid	-0.4618	-0.4669 to -0.4567	Yes	****	<0.0001			
PAmCherry1 plasmid vs. PAmCherry1 genome	0.002900	-0.001906 to 0.007707	No	ns	0.4073			
PATagRFP plasmid vs. PAmKate plasmid	-0.04286	-0.04698 to -0.03874	Yes	****	<0.0001			
PATagRFP plasmid vs. PAmCherry1 genome	0.4218	0.4180 to 0.4256	Yes	****	<0.0001			
PAmKate plasmid vs. PAmCherry1 genome	0.4647	0.4594 to 0.4699	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
PAmCherry1 plasmid vs. PATagRFP plasmid	1.487	1.906	-0.4189	0.001389	8848	6465	301.5	11735
PAmCherry1 plasmid vs. PAmKate plasmid	1.487	1.948	-0.4618	0.001982	8848	7269	233.0	15071
PAmCherry1 plasmid vs. PAmCherry1 genome	1.487	1.484	0.002900	0.001871	8848	6626	1.551	14833
PATagRFP plasmid vs. PAmKate plasmid	1.906	1.948	-0.04286	0.001604	6465	7269	26.71	9057
PATagRFP plasmid vs. PAmCherry1 genome	1.906	1.484	0.4218	0.001465	6465	6626	287.9	8630
PAmKate plasmid vs. PAmCherry1 genome	1.948	1.484	0.4647	0.002036	7269	6626	228.3	13848

Figure S3

Sample

	Neighboring	Target
n	8	8

To analyze statistical difference between the photoactivation trends, areas under the curves (AUCs) of photoactivation ratio (RFP/RFP_0) vs. time were determined for individual cells. Logged AUC values were used for the statistical analysis.

Shapiro-Wilk normality test

Shapiro-Wilk test	Neighboring	Target
W	0.9694	0.9613
P value	0.8935	0.8220
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

➔ Normality test was passed.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	1.842, 7, 7
P value	0.4389
P value summary	ns
Significantly different (P < 0.05)?	No

➔ Variances are not significantly different

Unpaired t test

Unpaired t test	
P value	0.0011
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4.108, df=14

➔ The logged AUCs values are significantly different.

Figure S4B

Human cell

Sample size

	Column A: Before photoactivation	Column B: After photoactivation
Neighboring	56 cells	56 cells

Ratio paired t test

Ratio paired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=38.26, df=55
Number of pairs	56
How big is the difference?	
Geometric mean of ratios (B / A)	50.43
SD of log(ratios)	0.3330
SEM of log(ratios)	0.04450
95% confidence interval	41.06 to 61.92
R squared (partial eta squared)	0.9638
How effective was the pairing?	
Correlation coefficient (r)	0.3877
P value (one tailed)	0.0016
P value summary	**
Was the pairing significantly effective?	Yes

Yeast

Sample size

	Column A: Before photoactivation	Column B: After photoactivation
Neighboring	96 cells	96 cells

Logged fluorescence values were used for statistical analysis.

Ratio paired t test

Ratio paired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=99.83, df=95
Number of pairs	96
How big is the difference?	
Geometric mean of ratios (B / A)	2.243
SD of log(ratios)	0.03443
SEM of log(ratios)	0.003514
95% confidence interval	2.207 to 2.279
R squared (partial eta squared)	0.9906
How effective was the pairing?	
Correlation coefficient (r)	0.6531
P value (one tailed)	<0.0001
P value summary	****
Was the pairing significantly effective?	Yes

➔ The logged fluorescence values are significantly different.

Figure S7

Figure S7A

Relative brightness

Sample size (cells)

	mGold	Ypet	mCitrine	mVenus(L46M;Q69M)	mVenus (L46F;I47V;C48L)	mVenus (Q204N;S205A;K206S)	mVenus (L46F;Q69M)	mVenus (L46F)	mVenus
Replicate 1	2543	6135	5886	4047	3407	3760	4542	3440	1752
Replicate 2	5648	3621	4257	5658	4061	3377	6430	5476	4938
Replicate 3	3853	4874	4686	4164	5557	5138	4645	4850	3208

For each replicate, the relative brightness of individual cells were determined and mean relative brightness was calculated (of the replicate). These mean relative brightness values were used for statistical analysis. n = 3 biological replicates.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	0.3007 (7, 16)
P value	0.9434
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

ANOVA summary

ANOVA summary	
F	37.30
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.9423

➔ The relative brightness values are significantly different.

Bonferroni's multiple comparisons test

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
mGold vs. mVenus	0.1903	-0.07917 to 0.4598	No	ns	0.2788			
mVenus(L46F;I47V;C48L) vs. mVenus	0.3047	0.03517 to 0.5742	Yes	*	0.0225			
mVenus(Q204N;S205A;K206S) vs. mVenus	-0.9074	-1.177 to -0.6379	Yes	****	<0.0001			
mVenus(L46F) vs. mVenus	0.06167	-0.2078 to 0.3312	No	ns	>0.9999			
mVenus(L46F;Q69M) vs. mVenus	-0.1963	-0.4658 to 0.07317	No	ns	0.2462			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
mGold vs. mVenus	2.167	1.977	0.1903	0.09227	3	3	2.063	16
mVenus(L46F;I47V;C48L) vs. mVenus	2.281	1.977	0.3047	0.09227	3	3	3.302	16
mVenus(Q204N;S205A;K206S) vs. mVenus	1.069	1.977	-0.9074	0.09227	3	3	9.834	16
mVenus(L46F) vs. mVenus	2.038	1.977	0.06167	0.09227	3	3	0.6683	16
mVenus(L46F;Q69M) vs. mVenus	1.780	1.977	-0.1963	0.09227	3	3	2.128	16

Photobleaching half-life

Sample size (cells)

	mGold	Ypet	mCitrine	mVenus(L46M;Q69M)	mVenus (L46F;I47V;C48L)	mVenus (Q204N;S205A;K206S)	mVenus (L46F;Q69M)	mVenus (L46F)	mVenus
Replicate 1	2543	6135	5886	4047	3407	3760	4542	3440	1752
Replicate 2	5648	3621	4257	5658	4061	3377	6430	5476	4938
Replicate 3	3853	4874	4686	4164	5557	5138	4645	4850	3208

For each replicate, the photobleaching half-lives of individual cells were determined and photobleaching half-life was calculated (of the replicate). These mean photobleaching half-life values were used for statistical analysis. n = 3 biological replicates.

Brown-Forsythe test

Brown-Forsythe test	
F (DFn, DFd)	1.560 (7, 16)
P value	0.2176
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

ANOVA summary

ANOVA summary	
F	171.7
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.9869

➔ The photobleaching half-life values are significantly different.

Bonferroni's multiple comparisons test

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
mGold vs. mVenus	77.24	66.59 to 87.89	Yes	****	<0.0001			
mVenus(L46F;I47V;C48L) vs. mVenus	37.85	27.20 to 48.50	Yes	****	<0.0001			
mVenus(Q204N;S205A;K206S) vs. mVenus	73.34	62.69 to 83.99	Yes	****	<0.0001			
mVenus(L46F) vs. mVenus	40.08	29.43 to 50.73	Yes	****	<0.0001			
mVenus(L46F;Q69M) vs. mVenus	-6.200	-16.85 to 4.450	No	ns	0.5421			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
mGold vs. mVenus	100.6	23.40	77.24	3.646	3	3	21.18	16
mVenus(L46F;I47V;C48L) vs. mVenus	61.25	23.40	37.85	3.646	3	3	10.38	16
mVenus(Q204N;S205A;K206S) vs. mVenus	96.74	23.40	73.34	3.646	3	3	20.11	16
mVenus(L46F) vs. mVenus	63.49	23.40	40.08	3.646	3	3	10.99	16
mVenus(L46F;Q69M) vs. mVenus	17.20	23.40	-6.200	3.646	3	3	1.700	16

Figure S7C

Sample size (cells)

	Column A: mVenus			Column B: mGold		
	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
Trial 1	1143	694	1341	2363	484	603
Trial 2	2429	2767	2088	4061	8341	4573

For each cell, the area under the curve of normalized fluorescence (F/F0) vs. time was calculated. For each replicate, the mean AUC was determined. Statistical analysis was performed on these mean AUCs.; n = 6 biological replicates.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	2.797, 5, 5
P value	0.2834
P value summary	ns

Significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Unpaired t test

Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=8.472, df=10

➔ The AUC values are significantly different.

Figure S7D

Sample size (cells)

	Column A: mVenus			Column B: mGold		
	replicate 1	replicate 2	replicate 3	replicate 1	replicate 2	replicate 3
Trial 1	183	206	152	219	197	208
Trial 2	165	161	140	216	267	164

For each cell, the area under the curve of normalized fluorescence (F/F0) vs. time was calculated. For each replicate, the mean AUC was determined. Statistical analysis was performed on these mean AUCs.; n = 6 biological replicates.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	3.360, 5, 5
P value	0.2095
P value summary	ns
Significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Unpaired t test

Unpaired t test	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=92.81, df=10
How big is the difference?	
Mean of column A	98.87
Mean of column B	228.4
Difference between means (B - A) ± SEM	129.5 ± 1.395
95% confidence interval	126.4 to 132.6
R squared (eta squared)	0.9988

➔ The AUC values are significantly different.

Figure S7E

Sample size (cells)

	Column A: mVenus	Column B: mGold
n	17	15

Shapiro-Wilk test

Shapiro-Wilk test	mVenus	mGold
W	0.9434	0.9742
P value	0.3603	0.9151
Passed normality test (alpha=0.05)?	Yes	Yes
P value summary	ns	ns

Normality test is passed.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	5.422, 14, 16
P value	0.0019
P value summary	**
Significantly different (P < 0.05)?	Yes

➔ The variances are significantly different.

Unpaired t test with Welch's correction

Unpaired t test with Welch's correction	
P value	<0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=17.64, df=18.50
How big is the difference?	
Mean of column A	40.55
Mean of column B	68.09
Difference between means (B - A) ± SEM	27.54 ± 1.561
95% confidence interval	24.27 to 30.81
R squared (eta squared)	0.9439

F test to compare variances

F test to compare variances	
F, DFn, Dfd	3.972, 2, 2
P value	0.4023
P value summary	ns
Significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Unpaired t-test

Unpaired t test	
P value	0.0006
P value summary	***
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=9.843, df=4

➔ The logged AUCs values are significantly different.

Figure S8G

Sample size (transfections)

	Column A: mVenus	Column B: mGold

n	3	3

n = 3 biological replicates.

Mann Whitney test

Mann Whitney test	
P value	0.1000
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	15 , 6
Mann-Whitney U	0
Difference between medians	
Median of column A	1.430, n=3
Median of column B	1.357, n=3
Difference: Actual	-0.07323
Difference: Hodges-Lehmann	-0.07323

➔ The cytotoxicity values are not significantly different.

Figure S8I

Sample size (cells)

Column A: mVenus			Column B: mGold		
Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
632	562	2454	407	598	4438

For each replicate, image analysis was conducted to determine the fluorescence intensities of individual cells and calculate the mean fluorescence intensity (of the replicate). Statistical analysis was performed on these mean fluorescence intensity values; n = 3 biological replicates.

F test to compare variances

F test to compare variances	
F, DFn, Dfd	1.555, 2, 2
P value	0.7829
P value summary	ns
Significantly different (P < 0.05)?	No

➔ The variances are not significantly different.

Unpaired t-test

P value	**0.3286**
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.2738, df=4
t, df	t=9.843, df=4

➔ The brightness values are not significantly different.