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1 A monogenic and fast-responding Light-Inducible Cre recombinase as
2 a novel optogenetic switch

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5 Hélène Duplus-Bottin¹, Martin Spichthy^{1,*}, Gérard Triqueneaux¹, Christophe Place², Philippe
6 Emmanuel Mangeot³, Théophile Ohlmann³, Franck Vittoz² and Gaël Yvert^{1,#}

7

8

9 1) Laboratory of Biology and Modeling of the Cell, Université de Lyon, Ecole Normale
10 Supérieure de Lyon, CNRS, UMR5239, Université Claude Bernard Lyon 1, 46 allée d'Italie
11 69007 Lyon, France.

12

13 2) Laboratory of Physics, Université de Lyon, Ecole Normale Supérieure de Lyon, CNRS,
14 UMR5672, Université Claude Bernard Lyon 1, 46 allée d'Italie 69007 Lyon, France.

15

16 3) CIRI-Centre International de Recherche en Infectiologie, Université Claude Bernard Lyon
17 1, Université de Lyon, Inserm, U1111, CNRS, UMR5308, Ecole Normale Supérieure de
18 Lyon, 69007, Lyon, France

19

20 *) Current address: Laboratoire d'Innovation Moléculaire et Applications, Site de Mulhouse –
21 IRJBD, 3 bis rue Alfred Werner, 68057 Mulhouse Cedex, France

22

23 #) corresponding author.

24

25 Contact Information:

26 Gaël Yvert

27 Laboratory of Biology and Modeling of the Cell,

28 Ecole Normale Supérieure de Lyon, CNRS, Université de Lyon

29 46 Allée d'Italie, Lyon, F-69007, France

30 Gael.Yvert@ens-lyon.fr

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33

34 ABSTRACT

35

36

37 Optogenetics enables genome manipulations with high spatiotemporal resolution,
38 opening exciting possibilities for fundamental and applied biological research. Here, we
39 report the development of LiCre, a novel light-inducible Cre recombinase. LiCre is made of a
40 single flavin-containing protein comprising the asLOV2 photoreceptor domain of *Avena*
41 *sativa* fused to a Cre variant carrying destabilizing mutations in its N-terminal and C-terminal
42 domains. LiCre can be activated within minutes of illumination with blue light, without the
43 need of additional chemicals. When compared to existing photoactivatable Cre recombinases
44 based on two split units, LiCre displayed faster and stronger activation by light as well as a
45 lower residual activity in the dark. LiCre was efficient both in yeast, where it allowed us to
46 control the production of β -carotene with light, and in human cells. Given its simplicity and
47 performances, LiCre is particularly suited for fundamental and biomedical research, as well as
48 for controlling industrial bioprocesses.

49

50 INTRODUCTION

51

52 The wealth of knowledge currently available on the molecular regulations of living
53 systems - including humans - largely results from our ability to introduce genetic changes in
54 model organisms. Such manipulations have been extremely informative because they can
55 unambiguously demonstrate causal effects of molecules on phenotypes. The vast majority of
56 these manipulations were made by first establishing a mutant individual - or line of
57 individuals - and then studying it. This classic approach has two limitations. First, the
58 mutation is present in all cells of the individual. This complicates the analysis of the
59 contribution of specific cells or cell-types to the phenotypic alterations that are observed at the
60 whole-organism level. Second, when a mutation is introduced long before the phenotypic
61 analysis, it is possible that the organism has "adapted" to it, either via compensatory
62 regulations or, in case of mutant lines maintained over multiple generations, by compensatory
63 mutations.

64

65 For these reasons, other approaches relying on site-specific recombinases were
66 developed to introduce specific mutations in a restricted number of cells of the organism, and
67 at a specific time. For instance, the Cre/LoxP system^{1,2} consists of two manipulations: a stable
68 insertion, in all cells, of foreign 34-bp DNA sequences called LoxP, and the expression of the
69 Cre recombinase in some cells only, where it modifies the DNA by catalyzing recombination
70 between the LoxP sites. The result is a mosaic animal - or plant, or colony of cells - where
71 chromosomal DNA has been rearranged in some cells only. Cre is usually introduced via a
72 transgene that is only expressed in the cells to be mutated. The location and orientation of
73 LoxP sites can be chosen so that recombination generates either a deletion, an inversion or a
74 translocation. Similar systems were developed based on other recombinases/recognition
75 targets, such as Flp/FRT³ or Dre-rox⁴. To control the timing of recombination, several
76 systems were made inducible. Tight control was obtained using recombinases that are inactive
77 unless a chemical ligand is provided to the cells. For example, the widely-used Cre-ERT
78 chimeric protein can be activated by 4-hydroxy-tamoxifen⁵. Other inducible systems rely on
79 chemical-induced dimerization of two halves of the recombinase. For example, the FKBP-
80 FRB split Cre system consists of two inactive proteins that can assemble in the presence of
81 rapamycin to form a functional recombinase complex⁶. Similar systems were reported that
82 rendered dimerization of the split Cre fragments dependent on phytohormones⁷. Although
83 powerful, these systems present some caveats: ligands are not always neutral to cells and can

84 therefore perturb the biological process under investigation; since they diffuse in tissues, the
85 control of activation is sometimes not precise enough in space and/or time; and the cost or
86 side-effects of chemical inducers can be prohibitive for industrial or biomedical applications.

87

88 More recently, several authors modified these dimerizing split recombinases to make
89 them inducible by light instead of chemicals. This presents several advantages because i) light
90 can be used with extreme spatiotemporal precision and high reproducibility, ii) when applied
91 at low energy, it is neutral to many cell types, and iii) it is very cheap and therefore scalable to
92 industrial processes. The dimerization systems that were used come from developments made
93 in optogenetics, where various light, oxygen or voltage (LOV) protein domains have been
94 used as photosensory modules to control transcription⁸, protein degradation⁹, dimerization¹⁰⁻¹²
95 or subcellular relocalization^{13,14}. LOV domains belong to the Per-Arnt-Sim (PAS)
96 superfamily found in many sensors. They respond to light via a flavin cofactor located at their
97 center. In the *asLOV2* domain, blue light generates a covalent bond between a carbon atom of
98 a flavin mononucleotide (FMN) cofactor and a cystein side chain of the PAS fold^{15,16},
99 resulting in a conformational change including the unfolding of a large C-terminal α -helical
100 region called the $J\alpha$ helix^{17,18}. Diverse optogenetics tools have been developed by fusing LOV
101 domains to functional proteins, in ways that made the $J\alpha$ folding/unfolding critical for
102 activity¹⁹. Among these tools are several photodimerizers that proved useful to control the
103 activity of recombinases. Taslimi *et al.*²⁰ reported blue-light dependent heterodimerization of
104 a split Cre recombinase using the CIB1-CRY2 dimerizers from the plant *Arabidopsis thaliana*
105 and others successfully used the nMag/pMag dimerizers derived from Vivid (VVD), a protein
106 of the fungus *Neurospora crassa*^{21,22}. A third system was based on dimerizers derived from
107 the chromophore-binding photoreceptor phytochrome B (PhyB) of *A. thaliana* and its
108 interacting factor PIF3. In this case, red light was used for stimulation instead of blue light,
109 but the system required the addition of an expensive chemical, the chromophore
110 phycocyanobilin²³.

111

112 An ideal inducible recombinase is one that ensures both low basal activity and high
113 induced activity, that is simple to implement, cheap to use and fast to induce. All dimerizing
114 split Cre systems have in common that two protein units must be assembled in order to form
115 one functional Cre. Thus, the probability of forming a functional recombination synapse -
116 which normally requires four Cre molecules - is proportional to the product of the two units'
117 cellular concentrations to the power of four. Split systems therefore strongly depend on the

118 efficient expression of their two different coding sequences, as previously reported²⁴. An
119 inducible system based on a single protein may avoid this limitation. Its implementation by
120 transgenesis would also be simpler, especially in vertebrates.

121

122 We report here the development of LiCre, a novel Light-Inducible Cre recombinase
123 that is made of a single flavin-containing protein. LiCre can be activated within minutes of
124 illumination with blue light, without the need of additional chemicals, and it shows extremely
125 low background activity in absence of stimulation as well as high induced activity. Using the
126 production of carotenoids by yeast as a case example, we show that LiCre and blue light can
127 be combined to control metabolic switches that are relevant to the problem of metabolic
128 burden in bioprocesses. We also report that LiCre can be used efficiently in human cells,
129 making it suitable for biomedical research. Since LiCre offers cheap and precise
130 spatiotemporal control of a genetic switch, it is amenable to numerous biotechnological
131 applications, even at industrial scales.

132 RESULTS

133

134 *The stabilizing N-ter and C-ter α -helices of the Cre recombinase are critical for its*
135 *activity*

136

137 A variety of optogenetic tools have been successfully developed based on LOV
138 domain proteins, which possess α -helices that change conformation in response to light²⁵. We
139 reasoned that fusing a LOV domain to a helical domain of Cre that is critical for its function
140 could generate a single protein with light-dependent recombinase activity. We searched for
141 candidate α -helices by inspecting the structure of the four Cre units complexed with two LoxP
142 DNA targets^{26,27} (Fig. 1a-b). Each subunit folds in two domains that bind to DNA as a clamp.
143 Guo *et al.* initially reported that helices αA and αE of the amino-terminal domain, as well as
144 helix αN of the C-terminal domain participate to inter-units contacts²⁷. This role of helix αN
145 was later confirmed by Ennifar *et al*²⁶. Contacts between αA and αE associate all four amino-
146 terminal domains (Fig. 1a) and contacts involving αN lock the four carboxy-terminal domains
147 in a cyclic manner (Fig. 1b). These helices were therefore good candidates for manipulating
148 Cre activity. We focused on αA and αN because their location at protein extremities was
149 convenient to design chimeric fusions.

150

151 We tested the functional importance of helices αA and αN by gradually eroding them.
152 We evaluated the corresponding mutants by expressing them in yeast cells where an active
153 Cre can excise a repressive DNA element flanked by LoxP sites, and thereby switch ON the
154 expression of a Green Fluorescent Protein (GFP) (Fig. 1c). After inducing the expression of
155 Cre mutants with galactose, we counted by flow cytometry the proportion of cells that
156 expressed GFP and we used this measure to compare recombinase activities of the different
157 mutants (Fig. 1d). As a control, we observed that the wild-type Cre protein activated GFP
158 expression in all cells under these conditions. Mutants lacking the last 2 or the last 3 carboxy-
159 terminal residues displayed full activity. In contrast, mutants lacking 4 or more of the C-ter
160 residues were totally inactive. This was consistent with a previous observation that deletion of
161 the last 12 residues completely suppressed activity²⁸. Our series of mutants showed that helix
162 αN is needed for activity and that its residue D341 is crucial. The role of this aspartic acid is
163 most likely to stabilize the complex: the tetramer structure indicates salt bridges between
164 D341 and residue R139 of the adjacent unit (Fig. 1e). Interestingly, E340 might have a similar
165 role by interacting with R192, although this residue was not essential for activity.

166 Biomolecular simulations using a simplistic force-field model showed that the free-energy
167 barrier for displacing the α N helix was much lower if E340 and D341 were replaced by
168 alanines (Fig. 1f). Consistent with this prediction, we observed that a double mutant E340A
169 D341A lost ~10% of activity (Fig. 1g). This mild (but reproducible) reduction of activity
170 suggested that the double mutation E340A D341A led to a fragilized version of Cre where
171 multimerization was suboptimal.

172

173 We also tested the functional importance of α -helix A, either in a normal context
174 where the C-terminal part of Cre was intact or where it carried the destabilizing E340A
175 D341A mutation (Fig. 1g). Deletion of residues 2-37, which entirely ablated helix A,
176 eliminated enzymatic activity (Fig. 1g). Very interestingly, the effect of shorter deletions
177 depended on the C-terminal context. When the C-terminus was wild-type, removing residues
178 2-21 (immediately upstream of helix A) had no effect and removing residues 2-28 (partial
179 truncation of α A) decreased the activity by ~10%. When the C-terminus contained the E340A
180 D341A mutation, deletions 2-21 and 2-28 were much more severe, reducing the activity by
181 12% and 80%, respectively. This revealed genetic interactions between the extremities of the
182 protein, which is fully consistent with a cooperative role of helices α A and α N in stabilizing
183 an active tetramer complex. From these observations, we considered that photo-control of Cre
184 activity might be possible by fusing α A and α N helices to LOV domain photoreceptors.

185

186 *Fusions of LOV domains to monogenic Cre confer light-inducible activity*

187

188 Our first strategy was to fuse the α N carboxy-terminal helix of Cre to the amino-
189 terminal cap of the LOV-domain of protein Vivid (VVD), a well-characterized photosensor
190 from *Neurospora crassa*^{12,29,30}. The resulting chimeric protein, which contained the full-
191 length Cre connected to VVD via four amino-acids, did not display light-dependent
192 recombinase activity (Supplementary Fig. S1). Our next strategy was based on a modified
193 version of the asLOV2 domain from *Avena sativa* which had been optimized by Guntas *et*
194 *al.*³¹. These authors used it to build an optogenetic dimerizer by fusing its J α C-ter helix to the
195 bacterial *SsrA* peptide. Instead, we fused J α to the α A amino-terminal helix of Cre. Using the
196 same GFP reporter system as described above for detecting *in-vivo* recombination in yeast, we
197 built a panel of constructs with various fusion positions and we directly quantified their
198 activity with and without blue-light illumination. All fusions displayed reduced activity in
199 both dark and light conditions as compared to wild-type Cre. Three constructs -

200 corresponding to fusions of asLOV2 to residues 19, 27 and 32 of Cre, respectively - displayed
201 higher activity after light stimulation. We recovered the corresponding plasmids from yeast,
202 amplified them in bacteria to verify their sequence and re-transformed them in yeast which
203 confirmed the differential activity between dark and light conditions for all three constructs
204 (Fig. 2a). Fusion at position 32 (named LOV2_Cre32) displayed the highest induction by
205 light, with activity increasing from 15% in dark condition to 50% after 30 minutes of
206 illumination. Although this induction was significant, a 15% activity of the non-induced form
207 remained too high for most applications. We therefore sought to reduce this residual activity,
208 which we did in two ways.

209

210 First, we randomized the residues located at the junction between asLOV2 and Cre.
211 We used degenerate primers and *in-vivo* recombination (see methods) to mutagenize
212 LOV2_Cre32 at these positions and we directly tested the activity of about 90 random clones.
213 Five of them showed evidence of low residual activity in the dark and we characterized them
214 further by sequencing and re-transformation. For all five clones, residual activity was indeed
215 reduced as compared to LOV2_Cre32, with the strongest reduction being achieved by an
216 isoleucine insertion at the junction position (Fig. 2b iv). However, this improvement was also
217 accompanied by a weaker induced activity and a larger variability between independent
218 assays.

219

220 As a complementary approach to reduce residual activity, we took advantage of the
221 above-described genetic interaction between N-ter truncations and C-ter mutations targeting
222 residues 340 and 341. We built another series of constructs where asLOV2 fusions to αA
223 helix were combined with the A340A341 double mutation. This approach yielded one
224 construct (LOV2_CreAA20), corresponding to fusion at position 20, which displayed a
225 residual activity that was undistinguishable from the negative control, and a highly-
226 reproducible induced activity of ~25% (Fig. 2c). We called this construct LiCre (for 'Light-
227 inducible Cre') and characterized it further.

228

229 *Efficiency and dynamics of LiCre photoactivation*

230

231 We placed LiCre under the expression of the P_{MET17} promoter and we tested various
232 illumination intensities and durations on cells that were cultured to stationary phase in
233 absence of methionine (full expression). Activity was very low without illumination and

234 increased with both the intensity and duration of light stimulation (Fig. 3a). The minimal
235 intensity required for stimulation was comprised between 0.057 and 1.815 mW/cm². The
236 highest activity (~65% of switched cells) was obtained with 90 minutes illumination at 36.3
237 mW/cm². Extending illumination to 180 minutes did not further increase the fraction of
238 switched cells. Remarkably, we observed that 2 minutes of illumination was enough to switch
239 5% of cells, and 5 minutes illumination generated 10% of switched cells (Fig. 3b).

240

241 We compared these performances with those of two previous systems that were both
242 based on light-dependent complementation of a split Cre enzyme. We constructed plasmids
243 coding for proteins CreN59-nMag and pMag-CreC60 described in Kawano *et al.*²¹ and
244 transformed them in our yeast reporter strain. Similarly, we constructed and tested plasmids
245 coding for the proteins CRY2^{L348F}-CreN and CIB1-CreC described in Taslimi *et al.*²⁰. All four
246 coding sequences were placed under the control of the yeast P_{MET17} promoter. We analyzed
247 the resulting strains as above after adapting light to match the intensity recommended by the
248 authors (1.815 mW/cm² for nMag/pMag and 5.45 mW/cm² for CRY2^{L348F}/CIB1). As shown
249 in Fig. 3c, we validated the photoactivation of nMag/pMag split Cre in yeast, where activity
250 increased about 4-fold following 90 minutes of illumination, but we were not able to observe
251 photoactivation of the CRY2^{L348F}/CIB1 split Cre system (Fig. 3d). In addition, the
252 photoactivation of nMag/pMag split Cre was not as fast as the one of LiCre, since 30 minutes
253 of illumination was needed to observe a significant increase of activity. This observation is
254 consistent with the fact that dimerization of split Cre, which is not required for LiCre, limits
255 the rate of formation of an active recombination synapse. Another difference was that, unlike
256 LiCre, nMag/pMag split Cre displayed a mild but significant background activity in absence
257 of illumination (~6% of switched cells) (Fig. 3c). Altogether, these results show that, at least
258 in the yeast cellular context, LiCre outperforms these two other systems in terms of
259 efficiency, rapidity and residual background activity.

260

261 To demonstrate the control of a biological activity by light, we built a reporter where
262 Cre-mediated excision enabled the expression of the *HIS3* gene necessary for growth in
263 absence of histidine. We cultured cells carrying this construct and expressing LiCre and we
264 spotted them at various densities on two HIS⁻ selective plates. One plate was illuminated
265 during 90 minutes while the other one was kept in the dark and both plates were then
266 incubated for growth. After three days, colonies were abundant on the plate that had been

267 illuminated and very rare on the control plate (Fig. 3e). LiCre can therefore be used to trigger
268 cell growth with light.

269

270 We then sought to observe the switch in individual cells. To do so, we replaced GFP
271 by mCherry in our reporter system, so that the excitation wavelength of the reporter did not
272 overlap with stimulation of LiCre. We expressed and stimulated LiCre (90min at 3.63
273 mW/cm²) in cells carrying this reporter and subsequently imaged them over time. As
274 expected, we observed the progressive apparition of mCherry signal in a fraction of cells (Fig.
275 3f-g).

276

277 Although convenient for high-throughput quantifications, reporter systems based on
278 the *de novo* production and maturation of fluorescent proteins require a delay between the
279 time of DNA excision and the time of acquisition. We wished to bypass this limitation and
280 directly quantify DNA recombination. For this, we designed oligonucleotides outside of the
281 region flanked by LoxP sites. The hybridization sites of these primers are too distant for
282 efficient amplification of the non-edited DNA template but, after Cre-mediated excision of
283 the internal region, these sites become proximal and PCR amplification is efficient (Fig. 3h).
284 We mixed known amounts of edited and non-edited genomic DNA and performed real-time
285 qPCR to build a standard curve that could be used to infer the proportion of edited DNA from
286 qPCR signals. After this calibration, we applied this qPCR assay on genomic DNA extracted
287 from cells collected immediately after different durations of illumination at moderate intensity
288 (3.63 mW/cm²). Results were in full agreement with GFP-based quantifications (Fig. 3i).
289 Excision of the target DNA occurred in a significant fraction of cells after only 2 minutes of
290 illumination, and we estimated that excision occurred in about 30% and 40% of cells after 20
291 and 40 minutes of illumination, respectively. To determine if DNA excision continued to
292 occur after switching off the light, we re-incubated half of the cells for 90 minutes in the dark
293 prior to harvest and genomic DNA extraction. The estimated frequency of DNA excision was
294 strikingly similar to the one measured immediately after illumination (Fig. 3j). We conclude
295 that the reversal of activated LiCre to its inactive state is very rapid in the dark (within
296 minutes).

297

298 The qPCR assay also allowed us to compare the efficiency of light-induced
299 recombination between cell populations in exponential growth or in stationary phase. This
300 revealed that LiCre photoactivation was about 4-fold more efficient in non-dividing cells (Fig.

301 3k). Although the reasons for this difference remain to be determined, this increase of LiCre
302 photoactivation at stationary phase makes it particularly suitable for bioproduction
303 applications, where metabolic switching is often desired after the growth phase (see
304 discussion).

305

306 *Model of LiCre photo-activation*

307

308 We built a structural model of LiCre to conceptualize its mode of activation (Fig. 4a).
309 We based this model on i) the available structure of the Cre tetramer complexed with its target
310 DNA²⁷, ii) the available structure of asLOV2 in its dark state³¹ and iii) knowledge that the $J\alpha$
311 helix of asLOV2 domains unfolds after light activation^{17,18}. From this model, we hypothesize
312 that LiCre photoactivation may occur via two synergistic effects. First, the domain asLOV2
313 likely prevents Cre tetramerization in the dark state simply because of its steric occupancy.
314 The unfolding of the $J\alpha$ helix in the light state may allow asLOV2 to liberate the
315 multimerizing interface. Second, because the $J\alpha$ helix of asLOV2 and the αA helix of Cre are
316 immediately adjacent, it is unlikely that both of them can fold simultaneously in their native
317 conformation. The unfolding of $J\alpha$ in the light state may therefore stimulate proper folding of
318 αA , and thereby allow αA to bind to the adjacent Cre unit. Structural *in vitro* studies of LiCre
319 itself will be needed to validate these predictions.

320

321 According to this model, there are two possible steps limiting the activation of LiCre
322 in any one individual cell: the conformational change of LiCre monomers and the assembly of
323 a functional recombination synapse. We sought to investigate whether one of these two steps
324 was predominantly limiting over the other. We did this by studying cells carrying both the
325 GFP (green) and the mCherry (red) reporters. If monomer activation is predominantly
326 limiting, then two populations of cells are expected in an illuminated culture: cells that have
327 activated enough LiCre molecules to form an active synapse will efficiently switch both
328 reporters, and cells that have not activated enough LiCre monomers will leave both reporters
329 intact and display no fluorescence. Conversely, if assembly of a functional recombination
330 synapse is predominantly limiting, then the probability that a cell switches one reporter
331 should be independent on what happens at the other reporter and the population will then
332 contain a significant proportion of cells displaying fluorescence in only one color. After
333 stimulation with 3.63 mW/cm² blue light for 180 min, one third of the cells had switched
334 only one of the two reporters (fluorescence in only one of the channels), ruling out the

335 possibility that monomer activation is solely limiting (Fig. 4b). However, the probability that
336 a reporter had switched depended on whether the other reporter had also switched. For
337 example, the proportion of green cells in the whole population (marginal probability to switch
338 the green reporter) was ~20%, but the proportion of green cells in the subpopulation of red
339 cells (conditional probability) was over 30%. Similarly, red cells were more frequent in the
340 subpopulation of green cells than in the whole population (Fig. 4b). These observations ruled
341 out the possibility that formation of a functional LiCre:DNA synaptic complex was solely
342 limiting. We conclude that neither monomer activation nor synapse formation is the sole rate-
343 limiting step *in vivo*.

344

345 *LiCre provides a light-switch for carotenoid production*

346

347 LiCre offers a way to change the activities of cells without adding any chemical to
348 their environment. This potentially makes it an interesting tool to address the limitations of
349 metabolic burden in industrial bioproduction (see discussion). We therefore tested the
350 possibility to use LiCre to control the production of a commercial compound with light.

351

352 Carotenoids are pigments that can be used as vitamin A precursors, anti-oxydants or
353 coloring agents, making them valuable for the food, agriculture and cosmetics industries³².
354 Commercial carotenoids are generally produced by chemical synthesis or extraction from
355 vegetables, but alternative productions based on microbial fermentations offer remarkable
356 advantages, including the use of low-cost substrates and therefore a high potential for
357 financial gains. Bioproduction of carotenoids from microbes has therefore received an
358 increasing interest. It can be based on microorganisms that naturally produce carotenoids³². It
359 is also possible to introduce recombinant biosynthesis pathways in host microorganisms,
360 which offers the advantage of a well-known physiology of the host and of optimizations by
361 genetic engineering. For these reasons, strategies were previously developed to produce
362 carotenoids in the yeast *S. cerevisiae*. Expressing three enzymes (*crtE*, *crtI* and *crtYB*) from
363 *Xanthophyllomyces dendrorhous* enabled *S. cerevisiae* to efficiently convert farnesyl
364 pyrophosphate (FPP) into β -carotene³³. FPP is naturally produced by *S. cerevisiae* from
365 Acetyl-CoA and serves as an intermediate metabolite, in particular for the production of
366 ergosterol which is essential for cellular viability (Fig. 5). Thus, and as for any bioproduction
367 consuming a cellular resource, this design is associated with a trade-off: redirecting FPP to β -
368 carotene limits its availability for ergosterol biosynthesis and therefore impairs growth; and

369 its consumption by the host cell can limit the flux towards the recombinant pathway. A
370 promising way to deal with this trade-off would be to favor the flux towards ergosterol during
371 biomass expansion and, after enough producer cells are obtained, to switch the demand in
372 FPP towards β -carotene. We therefore explored if LiCre could offer this possibility.

373

374 First, we tested if LiCre could allow us to switch ON the exogenous production of
375 carotenoids with light. If so, one could use it to trigger production at the desired time of a
376 bioprocess. We constructed a *S. cerevisiae* strain expressing only two of the three enzymes
377 required for β -carotene production. Expression of the third enzyme, a bifunctional phytoene
378 synthase and lycopene cyclase, was blocked by the presence of a floxed terminator upstream
379 of the coding sequence of the *crtYB* gene (Fig. 5b). Excision of this terminator should restore
380 a fully-functional biosynthetic pathway. As expected, this strain formed white colonies on
381 agar plates, but it formed orange colonies after transformation with an expression plasmid
382 coding for Cre, indicating that β -carotene production was triggered (Fig. 5c). To test the
383 possible triggering by light, we transformed this strain with a plasmid encoding LiCre and
384 selected several transformants, which we cultured and exposed - or not - to blue light before
385 spotting them on agar plates. The illuminated cultures became orange while the non-
386 illuminated ones remained white. Plating a dilution of the illuminated cell suspension yielded
387 a majority of orange colonies, indicating that LiCre triggered *crtYB* expression and β -carotene
388 production in a high proportion of plated cells (Fig. 5c). We quantified bioproduction by
389 dosing total carotenoids in cultures that had been illuminated or not. This revealed that 72
390 hours after the light switch the intracellular concentration of carotenoids had jumped from
391 background levels to nearly 200 μ g/g (Fig. 5d). Thus, LiCre allowed us to switch ON the
392 production of carotenoids by yeast using blue light.

393

394 We then tested if LiCre could allow us to switch OFF with light the endogenous
395 ergosterol pathway that competes with carotenoid production for FPP consumption. The first
396 step of this pathway is catalysed by the Erg9p squalene synthase. Given the importance of
397 FPP availability for the production of various compounds, strategies have been reported to
398 control the activity of this enzyme during bioprocesses, especially in order to reduce it after
399 biomass expansion³⁴⁻³⁶. These strategies were not based on light but derived from
400 transcriptional switches that naturally occur upon addition of inhibitors or when specific
401 nutrients are exhausted from the culture medium. To test if LiCre could offer a way to switch
402 ERG9 activity with light, we modified the *ERG9* chromosomal locus and replaced the coding

403 sequence by a synthetic construct comprising a floxed sequence coding for Erg9p and
404 containing a transcriptional terminator, followed by a sequence coding for the catalytic
405 domain of the 3-hydroxy3-methylglutaryl coenzyme A reductase (tHMG1) (Fig. 5e). This
406 design prepares *ERG9* for a Cre-mediated switch: before recombination, Erg9p is normally
407 expressed; after recombination, *ERG9* is deleted and the tHMG1 sequence is expressed to
408 foster the mevalonate pathway. Given that *ERG9* is essential for yeast viability in absence of
409 ergosterol supplementation³⁷, occurrence of the switch can be evaluated by measuring the
410 fraction of viable yeast cells prior and after the induction of recombination. When doing so,
411 we observed that expression of Cre completely abolished viability, regardless of illumination.
412 In contrast, cultures expressing LiCre were highly susceptible to light: they were fully viable
413 in absence of illumination and lost ~23% of viable cells after light exposure (Fig. 5f). Thus,
414 LiCre offers the possibility to abolish the activity of the yeast squalene synthase by exposing
415 cells to light.

416

417 *LiCre switch in human cells*

418

419 Beyond yeast, LiCre may also have a large spectrum of applications on multicellular
420 organisms. Therefore, we tested its efficiency in human cells. For this, we constructed a
421 lentiviral vector derived from the simian immunodeficiency virus (SIV) and encoding a
422 human-optimized version of LiCre with a nuclear localization signal fused to its N-terminus.
423 To quantify the efficiency of this vector, we also constructed a stable reporter cell line where
424 expression of a membrane-located mCherry fluorescent protein could be switched ON by
425 Cre/Lox recombination. We obtained this line by Flp-mediated insertion of a single copy of
426 the reporter construct into the genome of Flp-In™ 293 cells (Fig. 6a, see methods). Our assay
427 consisted of producing LiCre-encoding lentiviral particle, depositing them on reporter cells
428 for 24h, illuminating the infected cultures with blue light and, 28 hours later, observing cells
429 by fluorescence microscopy. As shown in Fig. 6b, mCherry expression was not detected in
430 non-infected reporter cells. In cultures that were infected but not illuminated, a few positive
431 cells were observed. In contrast, infected cultures that had been exposed to blue light
432 contained mostly positive cells. This demonstrated the efficiency of the vector and that LiCre
433 was poorly active unless cells were illuminated. LiCre can therefore be used to switch genetic
434 activities in human cells with blue light.

435

436

437 DISCUSSION

438

439 By performing a mutational analysis of the Cre recombinase and testing the activity of
440 various chimeric proteins involving Cre variants and LOV-domains, we have developed a
441 novel, single-protein, light-inducible Cre recombinase (LiCre). As compared to two
442 previously-existing systems relying on light-dependent dimerization of split Cre fragments,
443 LiCre displayed lower background activity in the dark as well as faster and stronger activation
444 by light. LiCre enabled us to use blue light to switch ON the production of carotenoids by
445 yeast and to inactivate the yeast squalene synthase. Using a lentiviral vector and human
446 reporter cells, we also showed that LiCre could be used as an optogenetic switch in
447 mammalian systems. We discuss below the properties of LiCre as compared to previously-
448 reported photo-activatable recombinases and the potential of LiCre for applications in the
449 field of industrial bioproduction.

450

451 *LiCre versus other photo-activatable recombinases*

452

453 Several tools already exist for inducing site-specific recombination with light. They
454 fall in two groups: those that require the addition of a chemical and those that are fully
455 genetically-encoded. The first group includes the utilization of photocaged ligands instead of
456 4-hydroxy-tamoxifen to induce the activity of Cre-ERT. This pioneering approach was
457 successful in cultured human cells³⁸ as well as fish³⁹ and mouse⁴⁰. Later, a more complex
458 strategy was developed that directly rendered the active site of Cre photoactivatable via the
459 incorporation of photocaged amino-acids⁴¹. In this case, cells were provided with non-natural
460 amino-acids, such as the photocaged tyrosine ONBY, and were genetically modified in order
461 to express three foreign entities: a specifically evolved pyrrolysyl tRNA synthetase, a
462 pyrrolysine tRNA_{CUA} and a mutant version of Cre where a critical amino-acid such as Y324
463 was replaced by a TAG stop codon. The tRNA synthetase/tRNA_{CUA} pair allowed the
464 incorporation of the synthetic amino-acid in place of the nonsense mutation and the resulting
465 enzyme was inactive unless it was irradiated with violet or ultraviolet light. This strategy
466 successfully controlled recombination in cultured human cells⁴¹ and zebrafish embryos⁴². We
467 note that it presents several caveats: its combination of chemistry and transgenes is complex
468 to implement, the presence of the tRNA synthetase/tRNA_{CUA} pair can generate off-target
469 artificial C-terminal tails in other proteins by bypassing natural stop codons, and
470 violet/ultraviolet light can be harmful to cells. More recently, a radically-different chemical

471 approach was proposed which consisted of tethering an active TAT-Cre recombinase to
472 hollow gold nanoshells⁴³. When delivered to cells in culture, these particles remained trapped
473 in intracellular endosomes. Near-infrared photostimulation triggered activity by releasing the
474 recombinase via nanobubble generation occurring on the particle surface. A fourth system is
475 based on the chromophore phycocyanobilin, which binds to the PhyB receptor of *A. thaliana*
476 and makes its interaction with PIF3 dependent on red light. Photostimulation of this
477 interaction was used to assemble split Cre units into a functional complex in yeast²³. A major
478 interest of these last two systems is to offer the possibility to use red light, which is less
479 harmful to cells than blue or violet light and better penetrates tissues. However, all these
480 strategies require to efficiently deliver chemicals to the target cells at the appropriate time
481 before illumination; and their underlying chemistry can be expensive, especially for
482 applications in the context of large volumes such as industrial bioprocesses.

483

484 Other systems, such as LiCre, do not need chemical additives because they are fully
485 genetically-encoded. To our knowledge, there are currently three such systems. One is based
486 on the sequestration of Cre between two large photo-cleavable domains⁴⁴. The principle of
487 light-induced protein cleavage is very interesting but its application to Cre showed important
488 limitations: a moderate efficiency (~30% of ON cells after the switch), the dependence on a
489 cellular inhibitory chaperone, and the need of violet light. The two other systems are the
490 CRY2/CIB1 and nMag/pMag split Cre, where photo-inducible dimerizers bring together two
491 halves of the Cre protein^{20,21}. An important advantage of LiCre over these systems is that it is
492 made of a single protein. The first benefit of this is simplicity. More efforts are needed to
493 establish transgenic organisms expressing two open reading frames (ORFs) as compared to a
494 single one. This is particularly true for vertebrate systems, where inserting several constructs
495 requires additional efforts for characterizing transgene insertion sites and conducting genetic
496 crosses. For this reason, in previous studies, the two ORFs of the split Cre system were
497 combined in a single construct, where they were separated either by an internal ribosomal
498 entry site or by a sequence coding a self-cleaving peptide^{20,21,45}. Although helpful, these
499 solutions have important limits: with an IRES, the two ORFs are not expressed at the same
500 level; with a self-cleaving peptide, cleavage of the precursor protein can be incomplete,
501 generating uncleaved products with unknown activity. This was the case for nMag/pMag split
502 Cre in mammalian cells, where a non-cleaved form at ~72 kDa was reported and where
503 targeted modifications of the cleavage sequence increased both the abundance of this non-
504 cleaved form and the non-induced activity of the system⁴⁵. The second benefit of LiCre being

505 a single protein is to avoid problems of suboptimal stoichiometry between the two protein
506 units, which was reported as a possible issue for CRY2/CIB1 split Cre²⁴. A third benefit is to
507 avoid possible intra-molecular recombination between the homologous parts of the two
508 coding sequences. Although not demonstrated, this undesired possibility was suspected for
509 nMag/pMag split Cre because its two dimerizers derive from the same sequence⁴⁵. The other
510 advantages of LiCre are its performances. In the present study, we used a yeast-based assay to
511 compare LiCre with split Cre systems. Unexpectedly, although we used the improved version
512 of the CRY2/CIB1 split Cre containing the CRY2-L348F mutation²⁰, it did not generate
513 photo-inducible recombination in our assay. This is unlikely due to specificities of the
514 budding yeast, such as improper protein expression or maturation, because the original
515 authors reported activity in this organism²⁰. We do not explain this result but it is consistent
516 with the observations of Kawano *et al.*²¹ who detected extremely low photoactivation of the
517 original version of the CRY2/CIB1 split Cre, and with the observations of Morikawa *et al.*⁴⁵
518 who reported that the induced activity of the CRY2-L348F/CIB1 system was low and highly
519 variable. In contrast, we validated the efficiency of nMag/pMag split Cre and so did other
520 independent laboratories^{7,46,47,45}. LiCre, however, displayed weaker residual activity than
521 nMag/pMag split Cre in the dark. Reducing non-induced activity is essential for many
522 applications where recombination is irreversible. Very recently, the nMag/pMag split Cre
523 system was expressed in mice as a transgene - dubbed PA-Cre3.0 - which comprised the
524 promoter sequence of the chicken beta actin gene (CAG) and synonymous modifications of
525 the original self-cleaving coding sequence. The authors reported that this strategy abolished
526 residual activity, and they attributed this improvement to a reduction of the expression level
527 of the transgene⁴⁵. It will therefore be interesting to introduce LiCre in mice with a similar
528 expression system and compare it to PA-Cre3.0. Importantly, LiCre also displayed higher
529 induced activity and a faster response to light as compared to nMag/pMag split Cre. This
530 strong response probably results from its simplicity, since the activation of a single protein
531 involves fewer steps than the activation of two units that must then dimerize to become
532 functional. In conclusion, LiCre is simpler and more efficient than previously-existing photo-
533 activatable recombinases.

534

535 *LiCre and industrial bioproduction*

536

537 With their capability to convert low-cost substrates into valuable chemicals, cultured
538 cells have become essential actors of industrial production. However, although metabolic

539 pathways can be rewired in favor of the desired end-product, the yields of bioprocesses have
540 remained limited by a challenging and universal phenomenon called metabolic burden. This
541 effect corresponds to the natural trade-off between the fitness of host cells and their efficiency
542 at producing exogenous compounds⁴⁸. Loss of cellular fitness is sometimes due to viability
543 issues - *e.g.* if the end-product is toxic to the producing cells - and sometimes simply to the
544 fact that resources are allocated to the exogenous pathway rather than to the cellular needs.
545 Reciprocally, satisfying the cellular demands can compromise the efficiency of exogenous
546 pathways. In the case of carotenoids production by yeast, metabolic burden was shown to be
547 substantial⁴⁹ (μ_{max} reduced by ~12%). This growth defect presumably involves competition
548 for FPP, which is consumed to produce carotenoids but which is also crucially needed by
549 cells to synthesize ergosterol, a major constituent of their membranes⁵⁰.

550

551 To avoid the limitations caused by metabolic burden, a desired solution is to
552 artificially control molecular activities so that they can first be chosen to maximize biomass
553 expansion and then be changed in favor of bioconversion. Technically, this can be achieved
554 by adding inducers or repressors of gene expression into the cell culture, such as lactose or
555 hormones, but these molecules are too expensive to be used at industrial scales. Current
556 solutions therefore rely on physiological changes in gene expression that occur in host cells
557 during the course of fermentation, especially at the end of biomass expansion⁵¹. For example,
558 expression of human recombinant proteins under the yeast P_{MET17} promoter can be repressed
559 by extracellular methionine during the growth phase and triggered later after methionine is
560 consumed^{52,53}. Although useful, such strategies relying on endogenous molecular regulations
561 have two important caveats. Ensuring their robustness requires strict control of physiological
562 parameters; and each strategy is specific to the host organism and fermentation conditions and
563 is therefore not transferable. Such limitations would be alleviated if one could cheaply control
564 an artificial and generic metabolic switch.

565

566 Using light as the inducer is attractive in this regard. It is physiologically neutral to
567 most non-photosynthetic organisms, it is extremely cheap and it can be controlled in real-time
568 with extreme accuracy and reproducibility. In addition, because algae are sometimes used as
569 producers, engineers have already designed efficient ways to bring light to bioreactors of
570 various scales⁵⁴⁻⁵⁶. Placing metabolic activities of producing cells under optogenetic control is
571 therefore a promising perspective and several developments have been made in this direction.
572 Using the EL222 optogenetic expression system, Zhao *et al.* applied a two-regimes yeast

573 fermentation with a continuous illumination that maintained ethanol metabolism during the
574 growth phase, followed by light pulses stimulating isobutanol production during the
575 bioconversion phase⁵⁷. The potential of optogenetics was also illustrated by Miliias-Argeitis *et*
576 *al.* who designed a feedback control of *E. coli* growth and used it to stabilize fermentation
577 performances against perturbations⁵⁸.

578

579 We anticipate that LiCre can provide an alternative approach because it offers the
580 possibility to induce irreversible genetic changes by a transient exposure to light. Applying
581 light stimulation transiently on cells could be simpler to implement than continuously
582 controlling light conditions in a bioreactor. By constructing appropriate Lox-based circuits,
583 genetic changes can be designed beforehand to cause the desired switch of metabolic
584 activities. The first switch that can be beneficial is the triggering of bioproduction itself. In
585 principle, switching ON any artificially-designed bioproduction at the appropriate time after
586 biomass expansion can avoid the cell-growth delays caused by metabolic burden. In the
587 results above, we used carotenoids production as an example to illustrate how LiCre can be
588 used to trigger bioproduction by transient illumination. To explore the potential gains on
589 production yields, proof of concept experiments can now be made using LiCre in strains,
590 media and fermentative conditions that are relevant to industrial processes.

591 The other switch that is often desired after biomass expansion is a reduction of the
592 cellular demands for metabolites that are critical precursors of the product of interest. For
593 example, reducing the activity of the yeast Erg9p squalene synthase is beneficial when
594 producing terpenes - and in particular carotenoids - because more FPP becomes available for
595 the pathway of interest. Previous efforts could reduce this activity by mutagenesis⁵⁹,
596 replacement of the native *ERG9* promoter⁶⁰ or destabilization of the Erg9p protein⁶¹. In
597 addition, several laboratories were able to implement a dynamic switch of *ERG9* activity
598 using conventional genetic rewiring. By placing expression of *ERG9* under the control of the
599 *P_{MET3}* promoter, Asadollahi *et al.*³⁴ and Amiri *et al.*⁶² could repress it by adding methionine to
600 the culture medium, thereby improving the production of sesquiterpenes and linalool,
601 respectively. For the production of artemisinin, Paddon *et al.*⁶³ used the *P_{CTR3}* promoter and
602 CuSO₄, a cheaper inhibitor than methionine. Other studies placed the expression of *ERG9*
603 under the control of the *P_{HXT1}* promoter, which is repressed when glucose becomes naturally
604 exhausted from the medium^{35,36,64}. In the present study, LiCre enabled us to inactivate *ERG9*
605 by a transient illumination. Although full inactivation of *ERG9* causes cell death and is
606 therefore not appropriate for industrial applications, our results show that it is possible to

607 change ERG9 activity at a desired time and using an external stimulus that is cheaper than
608 inhibitors. Rather than full inactivation, other LiCre-based strategies can now be designed to
609 switch from a full activity to a reduced and viable activity. For example, one could insert a
610 weak *erg9* allele at another genomic locus of our *lox-ERG9-lox* strain, so that gene deletion is
611 partially complemented after recombination.

612 Given these considerations, optogenetic switches - and LiCre in particular - may allow
613 industries to address the issue of metabolic burden by integrating lighting devices in
614 bioreactors and by building switchable producer cells.

615

616 In conclusion, LiCre provides a cheap, simple, low-background, highly-efficient and
617 fast-responding way to induce site-specific recombination with light. Given that it works in
618 both yeast and mammalian cells, it opens many perspectives from fundamental and
619 biomedical research to industrial applications.

620

621 METHODS

622

623 **Strains and plasmids.** Plasmids, strains and oligonucleotides used in this study are
624 listed in Supplementary Tables S1, S2 and S3 respectively. LiCre plasmids are available from
625 the corresponding author upon request.

626

627 **Yeast reporter systems.** We ordered the synthesis of sequence LoxLEULoxHIS
628 (Supplementary Text S1) from GeneCust who cloned the corresponding BamHI fragment in
629 plasmid pHO-poly-HO to produce plasmid pGY262. The P_{TEF} -loxP-KILEU2-STOP-loxP-
630 spHIS5 construct can be excised from pGY262 by NotI digestion for integration at the yeast
631 *HO* locus. This way, we integrated it in a *leu2 his3* strain, which could then switch from
632 LEU+ his- to leu- HIS+ after Cre-mediated recombination (Fig. 4e). To construct a GFP-
633 based reporter, we ordered the synthesis of sequence LEULoxGreen (Supplementary Text S1)
634 from GeneCust who cloned the corresponding NheI-SacI fragment into pGY262 to obtain
635 pGY407. We generated strain GY984 by crossing BY4726 with FYC2-6B. We transformed
636 GY984 with the 4-Kb NotI insert of pGY407 and obtained strain GY1752. To remove the
637 *ade2* marker, we crossed GY1752 with FYC2-6A and obtained strain GY1761. Plasmid
638 pGY537 targeting integration at the *LYS2* locus was obtained by cloning the BamHI-EcoRI
639 fragment of pGY407 into the BamHI, EcoRI sites of pIS385. Plasmid pGY472 was produced
640 by GeneCust who synthesized sequence LEULoxmCherry (Supplementary Text S1) and
641 cloned the corresponding AgeI-EcoRI insert into the AgeI,EcoRI sites of pGY407. We
642 generated GY983 by crossing BY4725 with FYC2-6A. We obtained GY2033 by
643 transformation of FYC2-6B with a 4-Kb NotI fragment of pGY472. We obtained GY2207 by
644 transformation of GY983 with the same 4-Kb NotI fragment of pGY472. To generate
645 GY2206, we linearized pGY537 with NruI digestion, transformed in strain GY855 and
646 selected a LEU+ Lys- colony (pop-in), which we re-streaked on 5-FoA plates for vector
647 excision by counter-selection of URA3 (pop-out)⁶⁵. Strain GY2214 was a diploid that we
648 obtained by mating GY2206 with GY2207.

649

650 **Yeast expression plasmids.** Mutations E340A D341A were introduced by GeneCust
651 by site-directed mutagenesis of pSH63, yielding plasmid pGY372. We generated the N-
652 ter Δ 21 mutant of Cre by PCR amplification of the P_{GAL1} promoter of pSH63 using primer
653 1L80 (forward) and mutagenic primer 1L71 (reverse), digestion of pSH63 by AgeI and co-
654 transformation of this truncated plasmid and amplicon in a *trp1 Δ 63* yeast strain for

655 homologous recombination and plasmid rescue. We combined the N-ter Δ 21 and the C-ter
656 E340A D341A mutations similarly, but with pGY372 instead of pSH63. We generated N-
657 ter Δ 28 and N-ter Δ 37 mutants, combined or not with C-ter E340A D341A mutations, by the
658 same procedure where we changed 1L71 by mutagenic primers 1L72 and 1L73, respectively.

659 To generate a Cre-VVD fusion, we designed sequence CreCVII (Supplementary Text
660 S1) where the Cre sequence from GENBANK AAG34515.1 was fused to the VVD-
661 M135IM165I sequence from Zoltowski *et al.*²⁹ via four additional residues (GGSG). We
662 ordered its synthesis from GeneCust, and we co-transformed it in yeast with pSH63
663 (previously digested by NdeI and Sall) for homologous recombination and plasmid rescue.
664 This generated pGY286. We then noticed an unfortunate error in AAG34515.1, which reads a
665 threonine instead of an asparagine at position 327. We cured this mutation from pGY286 by
666 site-directed mutagenesis using primers 1J47 and 1J48, which generated pGY339 which
667 codes for Cre-VVD described in Supplementary Fig. S1. We constructed mutant C-ter Δ 14 of
668 Cre by site-directed mutagenesis of pGY286 using primers 1J49 and 1J50 which
669 simultaneously cured the N327T mutation and introduced an early stop codon. Mutants C-
670 ter Δ 2, C-ter Δ 4, C-ter Δ 6, C-ter Δ 8, C-ter Δ 10, C-ter Δ 12 of Cre were constructed by GeneCust
671 who introduced early stop codons in pGY339 by site-directed mutagenesis.

672
673 To test LOV2_Cre fusions, we first designed sequence EcoRI-LovCre_chimJa-BstBI
674 (Supplementary Text S1) corresponding to the fusion of asLOV2 with Cre via an artificial α -
675 helix. This helix was partly identical to the J α helix of asLOV2 and partly identical to the α A
676 helix of Cre. This sequence was synthesized and cloned in the EcoRI and BstBI sites of
677 pSH63 by GeneCust, yielding pGY408. We then generated and directly tested a variety of
678 LOV2_Cre fusions. To do so, we digested pGY408 with BsiWI and MfeI and used this
679 fragment as a recipient vector; we amplified the Cre sequence from pSH63 using primer
680 1G42 as the reverse primer, and one of primers 1M42 to 1M53 as the forward primer (each
681 primer corresponding to a different fusion position); we co-transformed the resulting
682 amplicon and the recipient vector in strain GY1761, isolated independent transformants and
683 assayed them with the protocol of photoactivation and flow-cytometry described below. We
684 generated and tested a variety of LOV2_CreAA fusions by following the same procedure
685 where plasmid pGY372 was used as the PCR template instead of pSH63. A transformant
686 corresponding to LOV2_Cre32 and showing light-dependent activity was chosen for plasmid
687 rescue, yielding plasmid pGY415. A transformant corresponding to LOV2_CreAA20 was
688 chosen for plasmid rescue, yielding plasmid pGY416. Sanger sequencing revealed that the

689 fusion sequence present in pGY416 was QID instead of QIA at the peptide junction (position
690 149 on LiCre sequence of Supplementary Text S2). All further experiments on LiCre were
691 derived from the fusion protein coded by pGY416.

692

693 To introduce random residues at the peptide junction of LOV2_Cre32 (Fig. 2b), we
694 first generated pGY417 using the same procedure as for the generation of pGY415 but with
695 pSH47 instead of pSH63 as the PCR template so that pGY417 has a URA3 marker instead of
696 TRP1. We then ordered primers 1N24, 1N25 and 1N26 containing degenerate sequences, we
697 used them with primer 1F14 to amplify the Cre sequence of pSH63, we co-transformed in
698 strain GY1761 the resulting amplicons together with a recipient vector made by digesting
699 plasmid pGY417 with NcoI and BsiWI, and we isolated and directly tested individual
700 transformants with the protocol of photoactivation and flow-cytometry described below.
701 Plasmids from transformants showing evidence of reduced background were rescued from
702 yeast and sequenced, yielding pGY459 to pGY464.

703

704 To replace the P_{GALI} promoter of pGY416 by the P_{MET17} promoter, we digested it with
705 SacI and SpeI, we PCR-amplified the P_{MET17} promoter of plasmid pGY8 with primers 1N95
706 and 1N96, and we co-transformed the two products in yeast for homologous recombination,
707 yielding plasmid pGY466. We changed the promoter of pGY415 using exactly the same
708 procedure, yielding plasmid pGY465. We changed the promoter of pSH63 similarly, using
709 primer 1O83 instead of 1N96, yielding plasmid pGY502.

710

711 To express the nMag/pMag split Cre system in yeast, we designed sequence CreN-
712 nMag-NLS-T2A-NLS-pMag-CreCpartly (Supplementary Text S1) and ordered its synthesis
713 from GeneCust. The corresponding BglII fragment was co-transformed in yeast for
714 homologous recombination with pGY465 previously digested with BamHI (to remove
715 asLOV2 and part of Cre), yielding plasmid pGY488 that contained the full system. We then
716 derived two plasmids from pGY488, each one containing one half of the split system under
717 the control of the Met17 promoter. We obtained the first plasmid (pGY491, carrying the
718 TRP1 selection marker) by digestion of pGY488 with SfoI and SacII and co-transformation of
719 the resulting recipient vector with a PCR product amplified from pGY465 using primers
720 1O80 and 1O82. We obtained the second plasmid (pGY501, carrying the URA3 selection
721 marker) in two steps. We first removed the pMag-CreC part of pGY488 by digestion with
722 NdeI and SacII followed by Klenow fill-in and religation. We then changed the selection

723 marker by digestion with PfoI and KpnI and co-transformation in yeast with a PCR product
724 amplified from pSH47 with primers 1O77 and 1O89.

725

726 To express the CRY2^{L348F}/CIB1 split Cre system in yeast, we designed sequences
727 CIB1CreCter and CRY2CreNter and ordered their synthesis from GeneCust, obtaining
728 plasmids pGY526 and pGY527, respectively. To obtain pGY531, we extracted the synthetic
729 insert of pGY527 by digestion with BglII and we co-transformed it in yeast with the NdeI-
730 BamHI fragment of pGY466 for homologous recombination. To obtain pGY532, we
731 extracted the synthetic insert of pGY526 by digestion with BglII and we co-transformed it in
732 yeast with the SacI-BamHI fragment of pSH47 for homologous recombination.

733

734 To build a switchable strain for carotene production, we modified EUROSCARF
735 strain Y41388 by integrating a LoxP-KILEU2-T_{ADHI}-LoxP cassette immediately upstream the
736 CrtYB coding sequence of the chromosomally-integrated expression cassette described by
737 Verwaal *et al.*³³. This insertion was obtained by transforming Y41388 with a 6.6Kb BstBI
738 fragment from plasmid pGY559 and selecting a Leu⁺ transformant, yielding strain GY2247.
739 To obtain pGY559, we first deleted the crtE and crtI genes from YEplac195-YB_E_I³³ by
740 MluI digestion and religation. We then linearized the resulting plasmid with SpeI and co-
741 transformed it for recombination in a *leu2Δ* yeast strain with a PCR amplicon obtained with
742 primers 1P74 and 1P75 and template pGY407. After Leu⁺ selection, the plasmid was
743 recovered from yeast, amplified in bacteria and verified by restriction digestion and
744 sequencing.

745

746 We used CRISPR/Cas9 to build a switchable strain for squalene synthase. We cloned
747 the synthetic sequence gERG9 (Supplementary Text S1) in the BamHI-NheI sites of the
748 pML104 plasmid⁶⁶ so that the resulting plasmid (pGY553) coded for a gRNA sequence
749 targeting *ERG9*. This plasmid was transformed in GY2226 together with a repair-template
750 corresponding to a 4.2-Kb EcoRI fragment of pGY547 that contained LoxP-synERG9-T_{ADHI}-
751 LoxP with homologous flanking sequences. The resulting strain was then crossed with
752 Y41388 to obtain GY2236.

753

754 **Yeast culture media.** We used synthetic (S) media made of 6.7 g/L Difco Yeast
755 Nitrogen Base without Amino Acids and 2 g/L of a powder which was previously prepared by
756 mixing the following amino-acids and nucleotides: 1 g of Adenine, 2 g of Uracil, 2 g of

757 Alanine, 2 g of Arginine, 2 g of Aspartate, 2 g of Asparagine, 2 g of Cysteine, 2 g of
758 Glutamate, 2 g of Glutamine, 2 g of Glycine, 2 g of Histidine, 2 g of Isoleucine, 4 g of
759 Leucine, 2 g of Lysine, 2 g of Methionine, 2 g of Phenylalanine, 2 g of Proline, 2 g of Serine,
760 2 g of Threonine, 2 g of Tryptophane, 2 g of Tyrosine and 2 g of Valine. For growth in
761 glucose condition, the medium (SD) also contained 20 g/L of D-glucose. For growth in
762 galactose condition (induction of P_{GAL1} promoter), we added 2% final (20 g/L) raffinose and
763 2% final (20 g/L) galactose (SGalRaff medium). Media were adjusted to pH=5.8 by addition
764 of NaOH 1N before autoclaving at 0.5 Bar. For auxotrophic selections or P_{MET17} induction,
765 we used media where one or more of the amino-acids or nucleotides were omitted when
766 preparing S. For example, SD-W-M was made as SD but without any tryptophane or
767 methionine in the mix powder.

768

769 **Photoactivation and flow-cytometry quantification of recombinase activity.**

770 Unless mentioned otherwise, quantitative tests were done by flow-cytometry using yeast
771 reporter strain GY1761. For photoactivation, we used a PAUL apparatus from GenIUL
772 equipped with 460 nm blue LEDs. Using a NovaII photometer (Ophir[®] Photonics), we
773 measured that a 100% intensity on this apparatus corresponded to an energy of 36.3 mW/cm².
774 We used Zomei ND filters when we needed to obtain intensities that were not tunable on the
775 device. The yeast reporter strain was transformed with the plasmid of interest, pre-cultured
776 overnight in selective medium corresponding to conditions of transcriptional activation of the
777 plasmid-borne Cre construct (SGalRaff-W for P_{GAL1} plasmids, SD-W-M for P_{MET17} plasmids,
778 SD-W-U-M for split Cre systems) with no particular protection against ambient light. The
779 saturated culture was transferred to two 96-well polystyrene flat-bottom Falcon[®] sterile plates
780 (100 μ l per well) and one plate was illuminated at the indicated intensities while the other
781 plate was kept in the dark. After the indicated duration of illumination, cells from the two
782 plates were transferred to a fresh medium allowing expression of GFP but not cell division
783 (SD-W-H or SD-W-U-H, strain GY1761 being auxotroph for histidine) and these cultures
784 were incubated at 30°C for 90 minutes. Cells were then either analyzed immediately by flow
785 cytometry, or blocked in PBS + 1mM sodium azide and analyzed the following day.

786 We acquired data for 10,000 events per sample using a FACSCalibur (BD
787 Biosciences) or a MACSQuant VYB (Miltenyi Biotech) cytometer, after adjusting the
788 concentration of cells in PBS. We analyzed raw data files in the R statistical environment
789 (www.r-project.org) using custom-made scripts based on the flowCore package⁷⁰ from
790 bioconductor (www.bioconductor.org). We gated cells automatically by computing a

791 perimeter of (FSC-H, SSC-H) values that contained 40% of events (using 2D-kernel density
792 distributions). A threshold of fluorescent intensity (GFP or mCherry) was set to distinguish
793 ON and OFF cells (*i.e.* expressing or not the reporter). To do this, we included in every
794 experiment a negative control made of the reporter strain transformed with an empty vector,
795 and we chose the 99.9th percentile of the corresponding 4,000 fluorescent values (gated cells)
796 as the threshold.

797

798 **Quantification of fluorescence levels from microscopy images.** For Fig. 3g, we
799 segmented individual cells on bright field images using the ImageJ Lasso plugin. Then, we
800 measured on the fluorescence images the mean gray value of pixels in each segmented area,
801 providing single-cell measures of fluorescence. For each image, the background fluorescence
802 level was quantified from eight random regions outside of cells and with areas similar to
803 single cells. This background level was subtracted from the fluorescence level of each cell.

804

805 **Quantification of carotenoids from yeast.** We strictly followed the procedure
806 described in Verwaal *et al.*³³, which consists of mechanical cell lysis using glass beads,
807 addition of pyrogallol, KOH-based saponification, and extraction of carotenoids in hexane.
808 Quantification was estimated by optical absorption at 449 nm using a Biowave
809 spectrophotometer.

810

811 **Human reporter cell line.** We built a reporter construct for Cre-mediated
812 recombination in human cells based on Addgene's plasmids 55779, containing a membrane-
813 addressed mCherry sequence⁶⁷ (mCherry-Mem) and 51269, containing a zsGreen-based
814 reporter of Cre recombination⁶⁸. Re-sequencing revealed that 51269 did not contain three
815 terminator sequences but only one between the LoxP sites. We applied a multi-steps
816 procedure to i) restore three terminators, ii) replace zsGreen with mCherry-Mem and iii) have
817 the final reporter in a vector suitable for targeted single-site insertion. First, we inserted a
818 LoxP site between restriction sites NheI and HindIII of pCDNA5/FRT (Invitrogen) by
819 annealing oligonucleotides 1098 and 1099, digesting and cloning this adaptor with NheI and
820 HindIII, which yielded plasmid pGY519. Second, we replaced in two different ways the
821 zsGreen sequence of 51269 by the mCherry-Mem sequence of 55779: either by cloning a
822 SmaI-NotI insert from 55779 into EcoRV-NotI of 51269, yielding plasmid pGY520, or by
823 cloning a EcoRI-NotI from 55779 into EcoRI-NotI of 51269, yielding plasmid pGY521.
824 Third, we inserted the HindIII-NotI cassette of pGY520 into the HindIII-NotI sites of

825 pGY519, yielding plasmid pGY523. Fourth, we inserted the HindIII-NotI cassette of pGY521
826 into the HindIII-NotI sites of pGY519, yielding plasmid pGY524. Fifth, a HindIII-BamHI
827 fragment of pGY523 containing one terminator, and a BglII-EcoRI fragment of pGY524
828 containing another terminator were simultaneously cloned as consecutive inserts in the BglII-
829 EcoRI sites of 51269. Finally, the resulting plasmid was digested with HindIII and BamHI to
830 produce a fragment that was cloned into the HindIII-BglII sites of pGY524 to produce
831 pGY525.

832 To establish stable cell lines, Flp-In™ T-REx™ 293 cells were purchased from
833 Invitrogen (ThermoFisher) and transfected with both the Flp recombinase vector (pOG44,
834 Invitrogen) and pGY525. Selection of clonal cells was first performed in medium containing
835 300 µg hygromycin (Sigma). After two weeks, we identified foci of cell clusters, which we
836 individualized by transferring them to fresh wells. One of these clones was cultured for three
837 additional weeks with high concentrations of hygromycin (up to 400 µg) to remove
838 potentially contaminating negative cells. The resulting cell line was named T4-2PURE.

839

840 **Lentivirus construct and production.** A synthetic sequence was ordered from
841 Genecust and cloned in the HindIII-NotI sites of pCDNA3.1 (Invitrogen™ V79020). This
842 insert contained an unrelated additional sequence that we removed by digestion with BamHI
843 and XbaI followed by blunt-ending with Klenow fill-in. The resulting plasmid (pGY561)
844 encoded LiCre optimized for mammalian codon usage, in-frame with a N-ter located SV40-
845 NLS signal. This NLS-LiCre sequence was amplified from pGY561 using primers Sauci and
846 Flard (Table S3), and the resulting amplicon was cloned in the AgeI-HindIII sites of the
847 GAE0 Self-Inactivating Vector⁶⁹, yielding pGY577. Lentiviral particles were produced in
848 Gesicle Producer 293T cells (TAKARA ref 632617) transiently transfected by pGY577 (40%
849 of total DNA), an HIV-1 helper plasmid (45% of total DNA) and a plasmid encoding the
850 VSV-g envelope (15%) as previously described⁶⁹. Particle-containing supernatants were
851 clarified, filtered through a 0.45-µm membrane and concentrated by ultracentrifugation at
852 40,000 g before resuspension in 1xPBS (100 fold concentration).

853

854 **LiCre assay in human cells.** About 3×10^5 cells of cell line T4-2PURE were plated in
855 two 6-well plates. After 24 h, 100 µl of viral particules were added to each well. After another
856 24 h, one plate was illuminated with blue-light (460 nm) using the PAUL apparatus installed
857 in a 37°C incubator, while the other plate was kept in the dark. For illumination, we applied a
858 sequence of 20 min ON, 20 min OFF under CO₂ atmosphere, 20 min ON where ON

859 corresponded to 3.63 mW/cm² illumination. Plates were then returned to the incubator and,
860 after 28 hours, were imaged on an Axiovert135 inverted fluorescent microscope.

861

862 **Calculation of potential mean force (PMF).** We calculated the free-energy profile
863 (reported in Figure 1f) for the unbinding of the C-terminal α -helix in the tetrameric Cre-
864 recombinase complex²⁶ (PDB Entry 1NZZ) as follows. The software we used were: the
865 CHARMM-GUI server⁷¹ to generate initial input files; CHARMM version c39b1⁷² to setup
866 the structural models and subsequent umbrella sampling by molecular dynamics; WHAM,
867 version 2.0.9 (<http://membrane.urmc.rochester.edu/content/wham/>) to extract the PMF; and
868 VMD, version 1.9.2⁷³ to visualize structures. To achieve sufficient sampling by molecular
869 dynamics, we worked with a structurally reduced model system. We focused thereby only on
870 the unbinding of the C-terminal α -helix of subunit A (residues 334:340) from subunit F.
871 Residues that did not have at least one atom within 25 Å from residues 333 to 343 of subunit
872 A were removed including the DNA fragments. Residues with at least one atom within 10 Å
873 were allowed to move freely in the following simulations; the remaining residues were fixed
874 to their positions in the crystal structure. For the calculation of the double mutant A340A341
875 the corresponding residues were replaced by alanine residues. The systems were simulated
876 with the CHARMM22 force field (GBSW & CMAP parameter file) and the implicit solvation
877 model FACTS⁷⁴ with recommended settings for param22 (*i.e.*, cutoff of 12 Å for nonbonded
878 interactions). Langevin dynamics were carried out with an integration time-step of 2 fs and a
879 friction coefficient of 4 ps⁻¹ for non-hydrogen atoms. The temperature of the heat bath was set
880 to 310 K. The hydrogen bonds were constrained to their parameter values with SHAKE⁷⁵.

881 The PMF was calculated for the distance between the center of mass of the α -helix
882 (residues 334:340 of subunit A) and the center of mass of its environment (all residues that
883 have at least one atom within 5 Å of this helix). Umbrella sampling⁷⁶ was performed with 13
884 independent molecular dynamics simulations where the system was restrained to different
885 values of the reaction coordinate (equally spaced from 4 to 10 Å) using a harmonic biasing
886 potential with a spring constant of 20 kcal mol⁻¹ Å⁻¹ (GEO/MMFP module of CHARMM).
887 Note that this module uses a pre-factor of 1/2 for the harmonic potential (as in the case of the
888 program WHAM).

889 For each simulation the value of the reaction coordinate was saved at every time-step
890 for 30 ns. After an equilibration phase of 5ns, we calculated for blocks of 5 ns the PMF and
891 the probability distribution function along the reaction coordinate using the weighted

892 histogram analysis method⁷⁷. A total of 13 bins were used with lower and upper boundaries at
893 3.75 and 10.25 Å, respectively, and a convergence tolerance of 0.01 kcal/mol. Finally, we
894 determined for each bin its relative free energy $F_i = -kT \ln(\bar{p}_i)$ where k was the Boltzmann
895 constant, T the temperature (310 K) and \bar{p}_i the mean value of the probability of bin i when
896 averaged over the five blocks. The error in the F_i estimate was calculated with $\sigma_{F_i} =$
897 $kT \sigma_{\bar{p}_i} / \bar{p}_i$ where $\sigma_{\bar{p}_i}$ was twice the standard error of the mean of the probability. An offset
898 was applied to the final PMF so that its lowest value was located at zero.

899

900 **qPCR quantification of recombinase activity.** We grew ten colonies of strain
901 GY1761 carrying plasmid pGY466 overnight at 30°C in SD-L-W-M liquid cultures. The
902 following day, we used these starter cultures to inoculate 12 ml of SD-W-M medium at OD_{600}
903 = 0.2. When monitoring growth by optical density measurements, we observed that it was
904 fully exponential after 4 hours and until at least 8.5 hours. At 6.5 hours of growth, for each
905 culture, we dispatched 0.1 ml in 96-well plate duplicates using one column (8 wells) per
906 colony, we stored aliquots by centrifuging 1 ml of the cell suspension at 3300 g and freezing
907 the cell pellet at -20°C ('Exponential' negative control) and we re-incubated the remaining of
908 the culture at 30°C for later analysis at stationary phase. We exposed one plate (Fig. 4j
909 'Exponential' cyan samples) to blue light (PAUL apparatus, 460 nm, 3.63 mW/cm² intensity)
910 for 40 min while the replicate plate was kept in the dark (Fig. 4j 'Exponential' grey samples).
911 We pooled cells of the same column and stored them by centrifugation and freezing as above.
912 The following day, we collected 1 ml of each saturated, froze and stored cells as above
913 ('Stationary' negative control). We dispatched the remaining of the cultures in a series of 96-
914 well plates (0.1 ml/well, two columns per colony) and we exposed these plates to blue light
915 (PAUL apparatus, 460 nm, 3.63 mW/cm² intensity) for the indicated time (0, 2, 5, 10, 20 or
916 40 min). For each plate, following illumination, we collected and froze cells from 6 columns
917 (Fig. 4h samples) and we reincubated the plate in the dark for 90 min before collecting and
918 freezing the remaining 6 columns (Fig. 4i, x-axis samples). For genomic DNA extraction, we
919 pooled cells from 6 wells of the same colony (1 column), we centrifuged and resuspended
920 them in 280 µl in 50 mM EDTA, we added 20 µl of a 2 mg/ml Zymolyase stock solution
921 (SEIKAGAKU, 20 U/mg) to the cell suspension and incubated it for 1h at 37°C for cell wall
922 digestion. We then processed the digested cells with the Wizard Genomic DNA Purification
923 Kit from Promega. We quantified DNA on a Nanodrop spectrophotometer and used ~100,000
924 copies of genomic DNA as template for qPCR, with primers 1P57 and 1P58 to amplify the

925 edited target and with primers 1B12, 1C22 to amplify a control HMLalpha region that we
926 used for normalization. We ran these reactions on a Rotorgene thermocycler (Qiagen). This
927 allowed us to quantify the rate of excision of the floxed region as $N_{\text{Lox}} / N_{\text{Total}}$, where N_{Lox}
928 was the number of edited molecules and N_{Total} the total number of DNA template molecules.
929 To estimate N_{Lox} , we prepared mixtures of edited and non-edited genomic DNAs, at known
930 ratios of 0%, 0.5%, 1%, 5%, 10%, 50%, 70%, 90%, 100% and we applied (1P57,1P58) qPCR
931 using these mixtures as templates. This provided us with a standard curve that we then used to
932 convert Ct values of the samples of interest into N_{Lox} values. To estimate N_{Total} , we qPCR-
933 amplified the HMLalpha control region from templates made of increasing concentrations of
934 genomic DNA. We then used the corresponding standard curve to convert the Ct value of
935 HMLalpha amplification obtained from the samples of interest into N_{Total} values.

936

937

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939

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947

948 AUTHORS CONTRIBUTIONS

949

950 Constructed plasmids and strains, performed flow cytometry and yeast experiments:

951 H.D-B.

952 Performed qPCR and human cell experiments: G.T.

953 Performed PMF computations: M.S.

954 Designed and produced lentiviral vector: P.M., G.T.
955 Conceived, designed and supervised the study, analysed flow-cytometry data: G.Y.
956 Wrote the paper: GY.
957 Contributed material: C.P., F.V., T.O.

958

959 COMPETING INTERESTS

960

961 The authors declare the following competing interest: A patent application covering
962 LiCre and its potential applications has been filed. Patent applicant: CNRS; inventors: H el ene
963 Duplus-Bottin, Martin Spichty and Ga el Yvert.

964

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1151 FIGURE LEGENDS

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1154 **Figure 1. N-ter and C-ter α -helices of Cre are critical for activity. a-b)** Structure of
1155 the Cre tetramer complexed with DNA (PDB: 1NZZ). The four N-ter domains (a) interact via
1156 contacts between α -helices A (green) and E (orange) and the four C-ter domains (b) interact
1157 via α -helices N (magenta). **c)** Yeast reporter system to quantify Cre efficiency. The STOP
1158 element includes a selectable marker and a terminator sequence which prevents expression of
1159 the downstream GFP sequence. **d)** Activity of wild-type and C-ter mutants of Cre measured
1160 as the fraction of cells expressing GFP (mean +/- s.e.m, $n = 3$ independent transformants).
1161 Numbers denote the number of residues deleted from the C-ter extremity. 'Vect': expression
1162 plasmid with no insert. **e)** Blow-up of α N helix. **f)** Energetics of α N displacing (see
1163 methods). PMF: Potential of Mean Force (\pm error defined as σ_{F_i} in Methods). **g)** Activity of
1164 Cre mutants lacking N-terminal residues 2 to X, combined or not with the A340 A341 C-
1165 terminal mutation (mean +/- s.e.m, $n = 3$ independent transformants). X was 21, 28 or 37 as
1166 indicated. Arrows, significantly different from WT at $p < 0.05$ (t -test). *ns*, non significant.

1167

1168 **Figure 2. Monogenic LOV2-Cre fusions display photoactivatable recombinase**
1169 **activity. a)** Fusions with wild-type Cre. **b)** Variants of LOV2_Cre32 carrying the indicated
1170 mutations at the peptide junction. **c)** Fusion with Cre carrying the A340A341 double
1171 mutation. **a - c)** Numbers indicate the positions on the Cre peptidic sequence where asLOV2
1172 was fused. All bar plots show recombinase activity measured by flow-cytometry (mean \pm sem
1173 of the proportion of switched cells, $n = 5$ independent transformants) after galactose-induced
1174 expression of the fusion protein, followed (cyan) or not (grey) by illumination at 460 nm, 36.3
1175 mW/cm², for 30 minutes. *, ** and ***: significantly different between dark and light

1176 conditions at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (t -test). *n.s.*, non significant ($p >$
1177 0.05).

1178

1179 **Figure 3. Functional properties of LiCre. a-b)** Energy and time dependence. Yeast
1180 cells carrying the reporter system of Fig. 1c and expressing LiCre were grown to stationary
1181 phase and illuminated with blue light (460 nm, see methods) at indicated intensities, then
1182 incubated in non-dividing conditions and processed by flow cytometry (mean \pm sem, strain
1183 GY1761 transformed with pGY466); $n = 4$ and 3 colonies in (a) and (b), respectively.
1184 Illumination conditions varied either in intensity (a) or duration (b). p : significance from t -
1185 test ($n=3$). The fraction of ON cells observed at 0 min was not significantly higher than the
1186 fraction of ON GY1761 cells transformed with empty vector pRS314 ($p>0.05$). c) Yeast strain
1187 GY1761 was transformed with plasmids pGY491 and pGY501 to express the two proteins of
1188 the nMag/pMag split Cre system of Kawano *et al.*²¹. Cells were processed as in (b) with a
1189 light intensity that matched authors recommendations (1.815 mW/cm²). Neg: no illumination,
1190 cells containing empty vectors only. p : significance from t -tests ($n= 4$). d) Yeast strain
1191 GY1761 was transformed with plasmids pGY531 and pGY532 to express the two proteins of
1192 the CRY2^{L348F}/CIB1 split Cre system of Taslimi *et al.*²⁰. Cells were processed as in (b) with
1193 or without illumination for 90 min at an intensity matching authors recommendations (5.45
1194 mW/cm²). Neg: no illumination, cells containing empty vectors only. e) Yeast cells
1195 expressing LiCre from plasmid pGY466 and carrying an integrated reporter conferring
1196 prototrophy to histidine were spotted on two His- plates at decreasing densities. Prior to
1197 incubation at 30°C, one plate (right) was illuminated for 90 minutes at 3.63 mW/cm²
1198 intensity. f) Time-lapse imaging of yeast cells expressing LiCre and carrying a similar
1199 reporter as Fig. 1c but where GFP was replaced by mCherry (strain GY2033 with plasmid
1200 pGY466). Cells were grown to stationary phase, illuminated for 90 min at 3.63 mW/cm²

1201 intensity, immobilized on bottom-glass wells in dividing condition and imaged at the
1202 indicated time. Bar: 10 μm . **g**) Quantification of intra-cellular mCherry fluorescence from (f),
1203 $n = 22$ cells. **h**) Design of qPCR assay allowing to quantify recombination efficiency. **i**)
1204 Quantification of excision by qPCR immediately after illumination at 3.63 mW/cm^2 intensity
1205 (stationary phase, strain GY1761 transformed with pGY466). **j**) DNA excision does not occur
1206 after illumination. X-axis: same data as shown on Y-axis in (i). Y-axis: same experiment but
1207 after illumination, cells were incubated for 90 minutes in dark and non-dividing condition
1208 prior to harvest and qPCR. **k**) Quantification of DNA excision by qPCR on exponentially-
1209 growing or stationary-phase cells (strain GY1761 transformed with pGY466) illuminated at
1210 3.63 mW/cm^2 intensity. Grey: no illumination. Bars in (i-k): s.e.m. ($n=10$ colonies).

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1212 **Figure 4. Model of LiCre activation. a)** The model was built using PDB structures
1213 1NZB (Cre) and 4WF0 (asLOV2). Green: residues of αA helix from Cre. Blue: residues of $\text{J}\alpha$
1214 helix from asLOV2. **b)** Two-colors switch assay. Yeast cells carrying LiCre and both GFP
1215 and mCherry reporters (strain GY2214 with plasmid pGY466) were grown to stationary
1216 phase in SD-M-W and illuminated for 180 min at 3.63 mW/cm^2 intensity. Cells were then
1217 incubated in the dark in non-dividing conditions and processed by flow-cytometry. Density
1218 plot (middle): fluorescent intensities for one sample. Barplot (right): mean \pm s.e.m. ($n=3$
1219 colonies) fraction of switched cells in the whole population (Green, Red, Red and green) or in
1220 the subpopulation of cells that also switched the other reporter (Green among red, Red among
1221 green). Grey bars: controls without illumination.

1222

1223 **Figure 5. Switching ON carotenoid production with light. a)** β -carotene
1224 biosynthetic pathway. Exogeneous genes from *X. dendrorhous* are printed in red. FPP,
1225 farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate. **b)** Scheme of the switchable

1226 locus of yeast GY2247. **c)** Photoswitchable bioproduction. Strain GY2247 was transformed
1227 with either pRS314 (Vect.), pGY466 (LiCre) or pGY502 (CreWT). Cells were cultured
1228 overnight in SD-M-W and the cultures were illuminated (460 nm, 90 min, 36.3 mW/cm²) or
1229 not and then spotted on agar plates. A and B correspond to two independent transformants of
1230 the LiCre plasmid. Colonies on the right originate from the illuminated LiCre (A) culture. **d)**
1231 Quantification of carotenoids production. Three colonies of strain GY2247 transformed with
1232 LiCre plasmid pGY466 were cultured overnight in SD-W-M. The following day, 10 ml of
1233 each culture were illuminated as in c), while another 10 ml was kept in the dark. These
1234 cultures were then incubated for 72 h at 30°C. Cells were pelleted (colors of the cell pellets are
1235 shown on picture) and processed for quantification (see methods). Units are micrograms of
1236 total carotenoids per gram of biomass dry weight. Bars: mean +/- s.e.m, $n = 3$. **e)** Scheme of
1237 the switchable locus of yeast GY2236. **f)** Light-induced deletion of squalene synthase gene.
1238 Strain GY2236 was transformed with either pRS314 (Vect), pGY502 (Cre) or pGY466
1239 (LiCre). Cells were cultured overnight in 4ml of SD-M-W. A 100- μ l aliquot of each culture
1240 was illuminated (as in c) while another 100- μ l aliquot was kept in the dark. A dilution at ~ 1
1241 cell/ μ l was then plated on SD-W. Colonies were counted after 3 days. *cfu*: colony forming
1242 units (mean +/- sem, $n \geq 3$ plates). **: $p < 0.01$ (*t*-test).

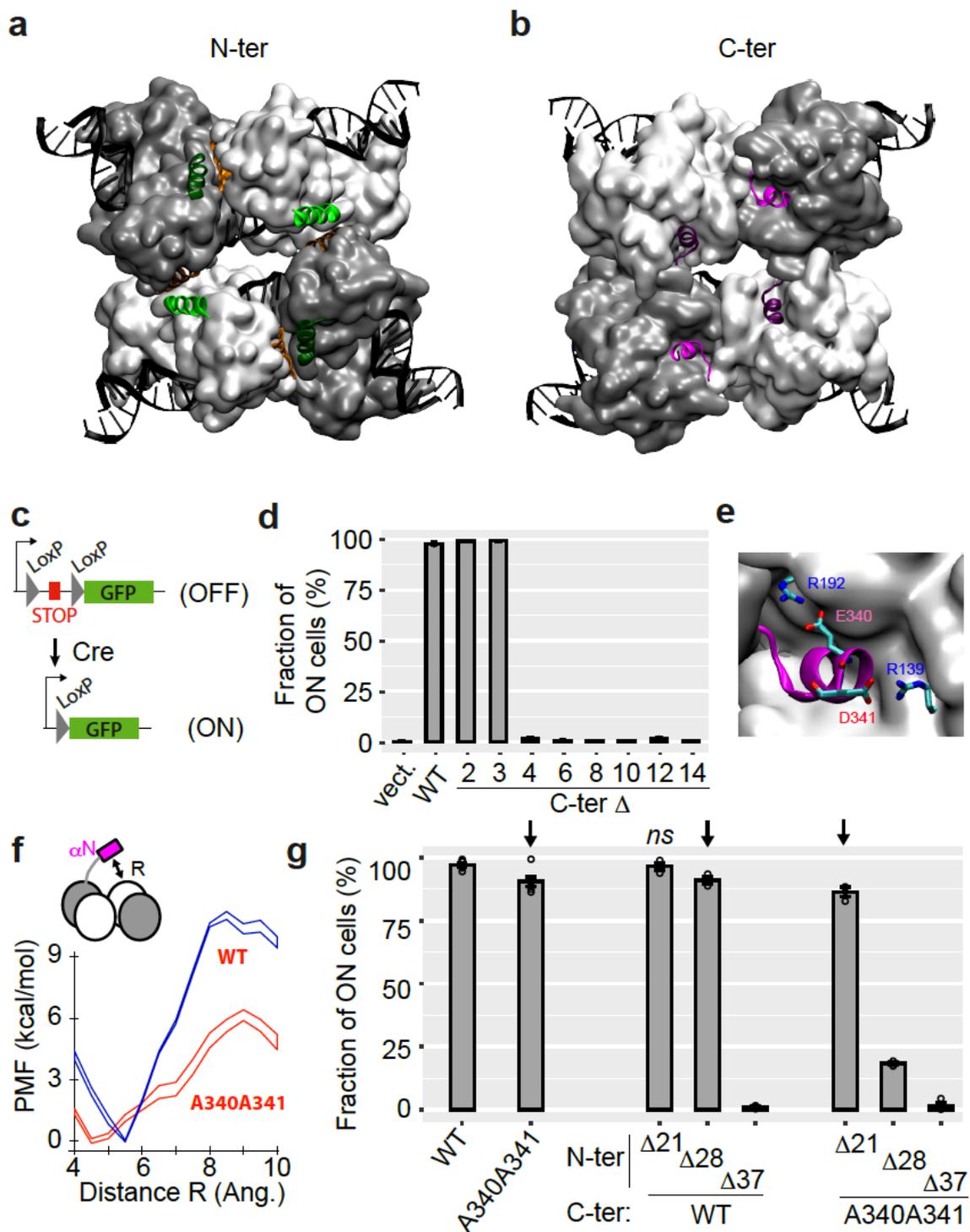
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1244 **Figure 6. LiCre photoactivation in human cells. a)** Left: Lentiviral SIN vector for
1245 LiCre expression (plasmid pGY577). *P_{CMV}*, early cytomegalovirus promoter; SIN, LTR
1246 regions of Simian Immunodeficiency Virus comprising a partially-deleted 3' U3 region
1247 followed by the R and U5 regions; *psi*, retroviral psi RNA packaging element; *cPPT* and *PPT*,
1248 central and 3' polypurine tracks, respectively; *RRE*, Rev/Rev-responsive element; *SA*, SIV
1249 Rev/Tat splice acceptor; *NLS*, nuclear localization signal; *WPRE*, woodchuck hepatitis virus
1250 regulatory element; *Helper*, plasmid coding for *gag*, *pol*, *tat* and *rev*; *VSV-g*, plasmid

1251 encoding the envelope of the vesicular stomatitis virus. Co-transfection in HEK293T cells
1252 produces pseudotyped particles. These particles are deposited on T4-2PURE reporter cells
1253 which are then illuminated and imaged. Right, genomic reporter locus of T4-2PURE cells.
1254 *P_{SV40}*, Promoter from SV40; *FRT*, FLP recognition targets; *Hyg^R*, hygromycin resistance; *pA*,
1255 poly-adenylation signal from SV40; *Zeo^R*, zeomycin resistance. Recombination between LoxP
1256 sites switches ON the expression of mCherry by removing three pA terminators. **b)**
1257 Microscopy images of T4-2PURE cells following the assay. Bars, 150 μ m. All three
1258 fluorescent frames were acquired at the same intensity and exposure time. Illumination
1259 corresponded to two 20 min exposures at 3.63 mW/cm², separated by 20 minutes without
1260 illumination.
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Figure 1

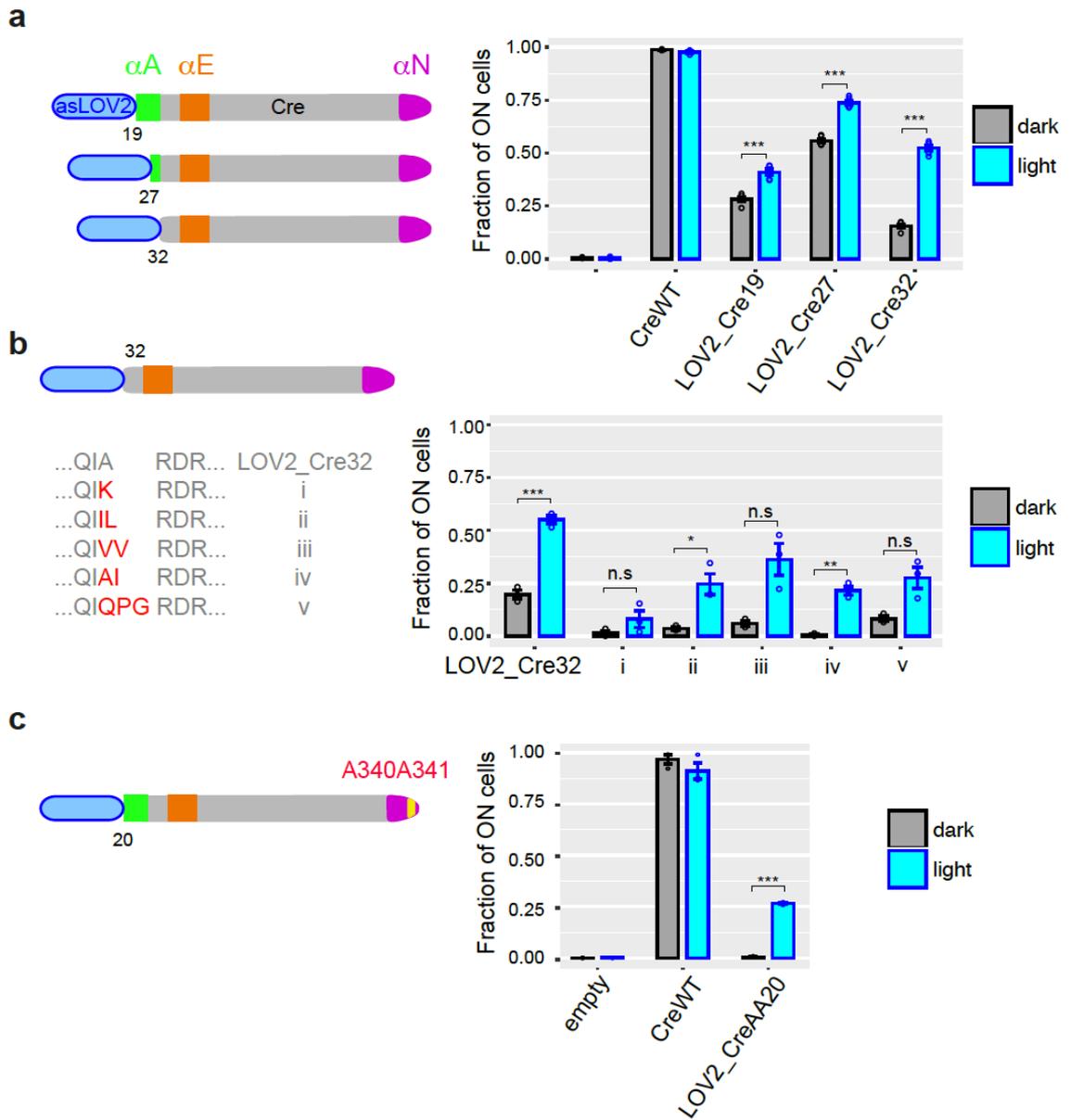


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Figure 2

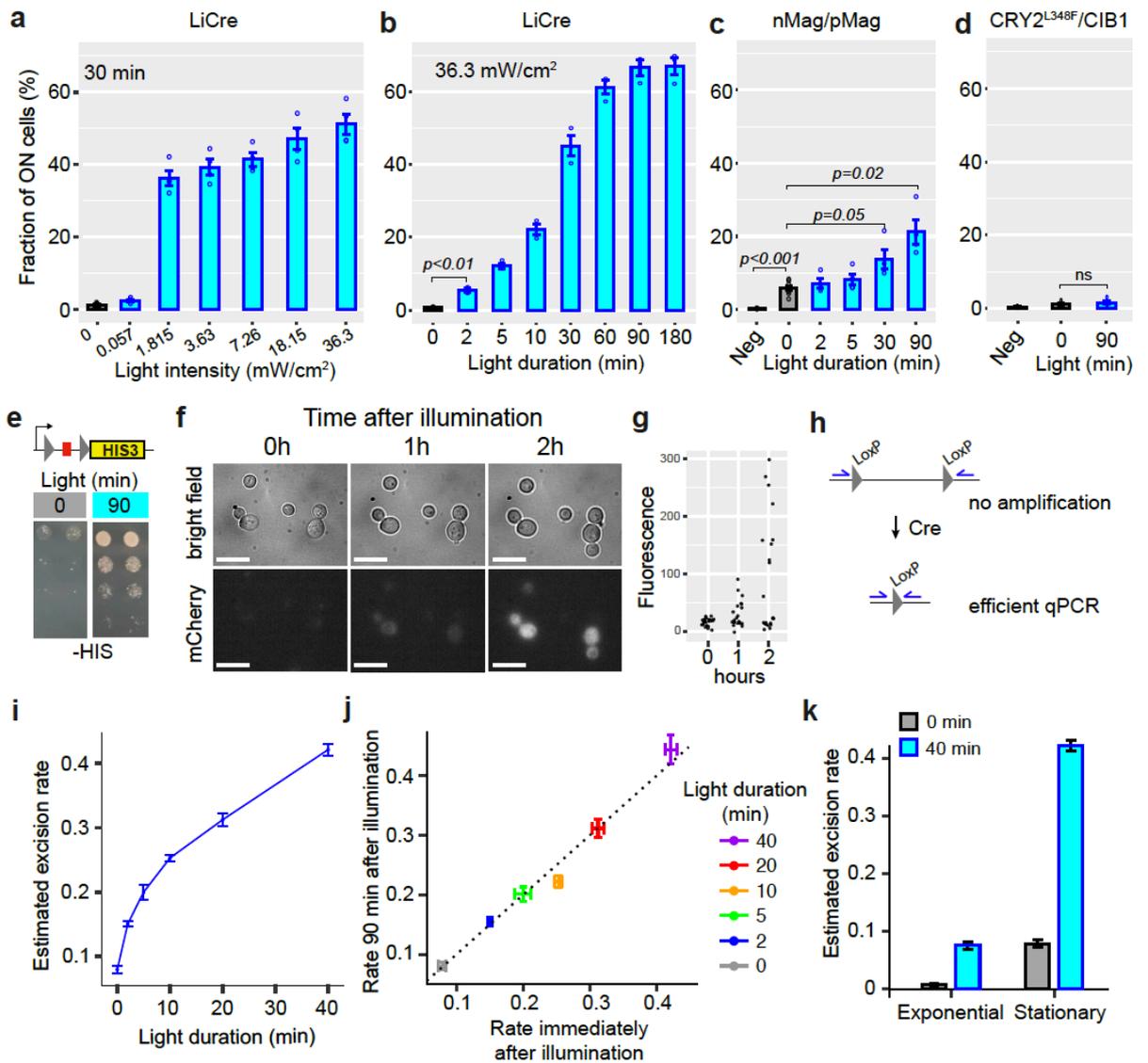


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Figure 3

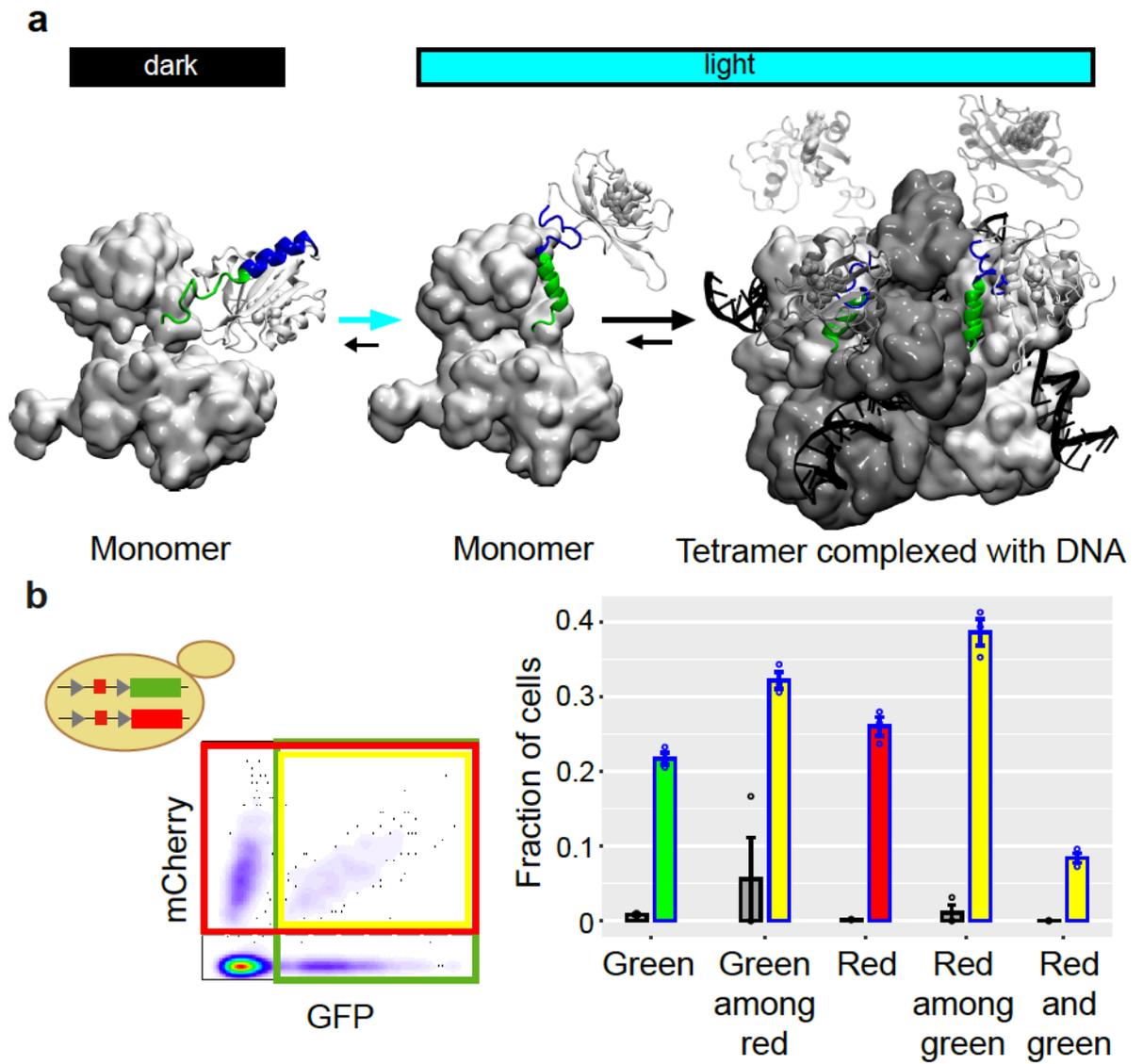


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Figure 4



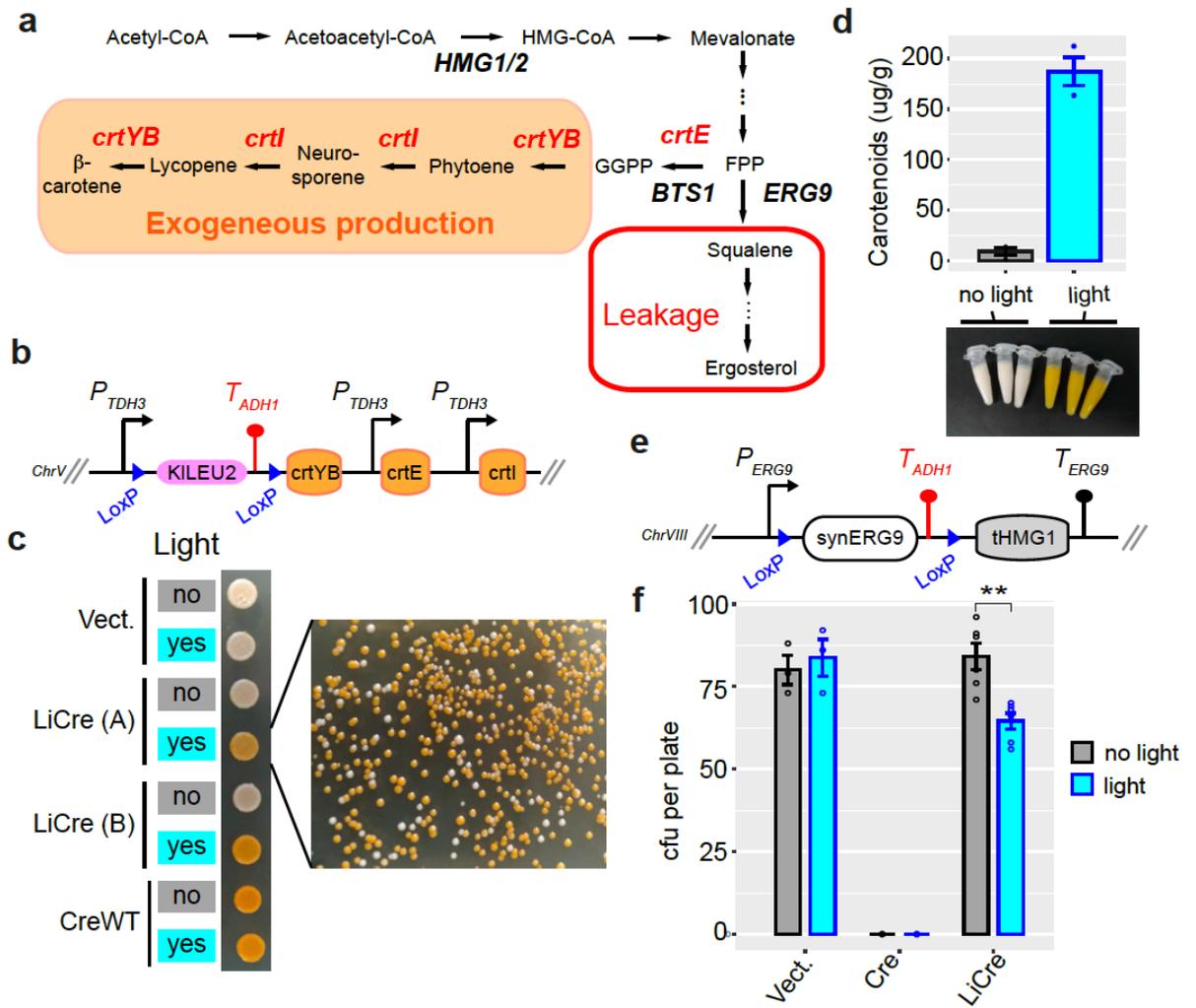
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Figure 5

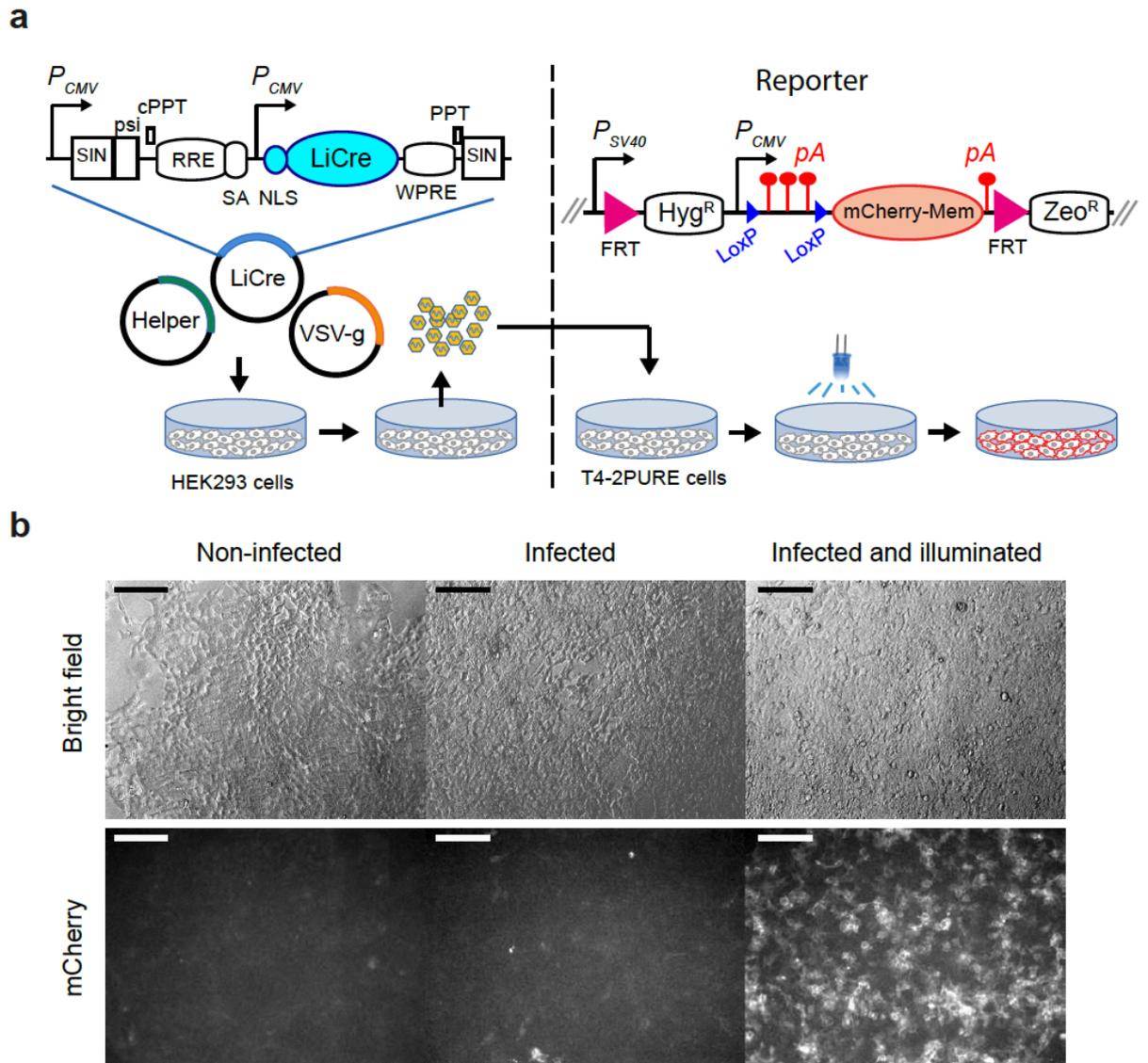


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Figure 6



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