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SUPPLEMENTARY INFORMATION

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Light-based control of metabolic flux through assembly of synthetic organelles

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Supplementary Materials for

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Supplementary Tables

Supplementary Table 1: Plasmids constructed for this study.

Plasmid	Position 1*	Position 2*	Position 3*	Marker	Vector type
pJLA121[0301]	P_{PGK1}_Multiple Cloning Sequence (MCS)_T_{CYC1}	EMPTY	EMPTY	*URA3*	2μ
pYZ12-B	EMPTY	EMPTY	EMPTY	*HIS3*	Integration into *HIS3* Locus
pYZ23	EMPTY	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNS1	P_{ADH1}_*FUS^N_Fusion Red_Cry2WT*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNS2	P_{ADH1}_*FusionRed_ Cry2WT*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNS3	P_{ADH1}_*FUS^N_Fusion Red_Cry2Olig*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNS4	P_{ADH1}_*FusionRed_ Cry2Olig*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNS5	P_{TEF1}_*VioB*_T_{ACT1}	P_{PGK1}_*VioA*_ T_{CYC1}	EMPTY	*HIS3*	Integration into *HIS3* Locus
pNS6	P_{TEF1}_*VioB*_T_{ACT1}	P_{PGK1}_*VioA*_ T_{CYC1}	P_{GPD}_*VioE*_T_{ADH1}	*HIS3*	Integration into *HIS3* Locus
pNS7	P_{TEF1}_*VioC*_T_{ACT1}	EMPTY	EMPTY	*URA3*	2μ
pNS8	P_{TEF1}_*VioE*_T_{ACT1}	EMPTY	EMPTY	*URA3*	CEN6_ARS4
pNS9	P_{TEF1}_*VioC*_T_{ACT1}	EMPTY	EMPTY	*URA3*	CEN6_ARS4
pNP1	P_{ADH1}_*FUS^N_Citrine _Cry2Olig_VioC*_ T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNP2	P_{ADH1}_ *VioC_FUS^N_Citrine_ Cry2Olig*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNP3	P_{ADH1}_*FUS^N_Fusion Red_Cry2Olig_VioE* _T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites

pNP4	P_{ADH1}_ *VioE_FUS*^{N}_ *FusionRed_Cry2Olig* _T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNP1-Drop	P_{ADH1}_*FUS*^{N}_*Citrine_Cry2_VioC*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNP2-Drop	P_{ADH1}_ *VioC_FUS*^{N}_ *Citrine_Cry2*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNP3-Drop	P_{ADH1}_*FUS*^{N}_*Fusion Red_Cry2_VioE*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
pNP7	P_{ADH1}_*FR_Cry2Olig _ VioE*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
EZ-L176	P_{GPD1}_*FUS*^{N}_*Fusion Red_Cry2*_ T_{ACT1}	EMPTY	EMPTY	*URA3*	2μ
EZ-L477	P_{ADH1}_*Citrine_Cry2 Olig_VioC*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
EZ-L498	P_{PGK1}_*FUS*^{N}_*Citrine _PixE*_T_{CYC1}	EMPTY	EMPTY	*HIS3*	Integration into *HIS3* Locus
EZ-L499	P_{ADH1}_*FUS*^{N}_*Fusion Red_PixD*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
EZ-L526	P_{PGK1}_*FUS*^{N}_*Citrine _PixE_VioE*_T_{CYC1}	EMPTY	EMPTY	*HIS3*	Integration into *HIS3* Locus
EZ-L527	P_{ADH1}_*FUS*^{N}_*Fusion Red_PixD_VioC*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
EZ-L528	P_{TEF1}_*VioB*_T_{ACT1}	P_{PGK1}_*VioA*_ T_{CYC1}	EMPTY	*LEU2*	Integration into *LEU2* Locus
EZ-L767	P_{ADH1}_*sFUS_Fusion Red_PixD*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
EZ-L786	P_{ADH1}_*sFUS_ PixD_VioC*_T_{ACT1}	EMPTY	EMPTY	Zeocin	Integration into δ-sites
EZ-L859	P_{PGK1}_*VioD*_T_{CYC1}	EMPTY	EMPTY	*URA3*	2μ

All vectors are constructed according to the map shown in Supplementary Fig. 11.

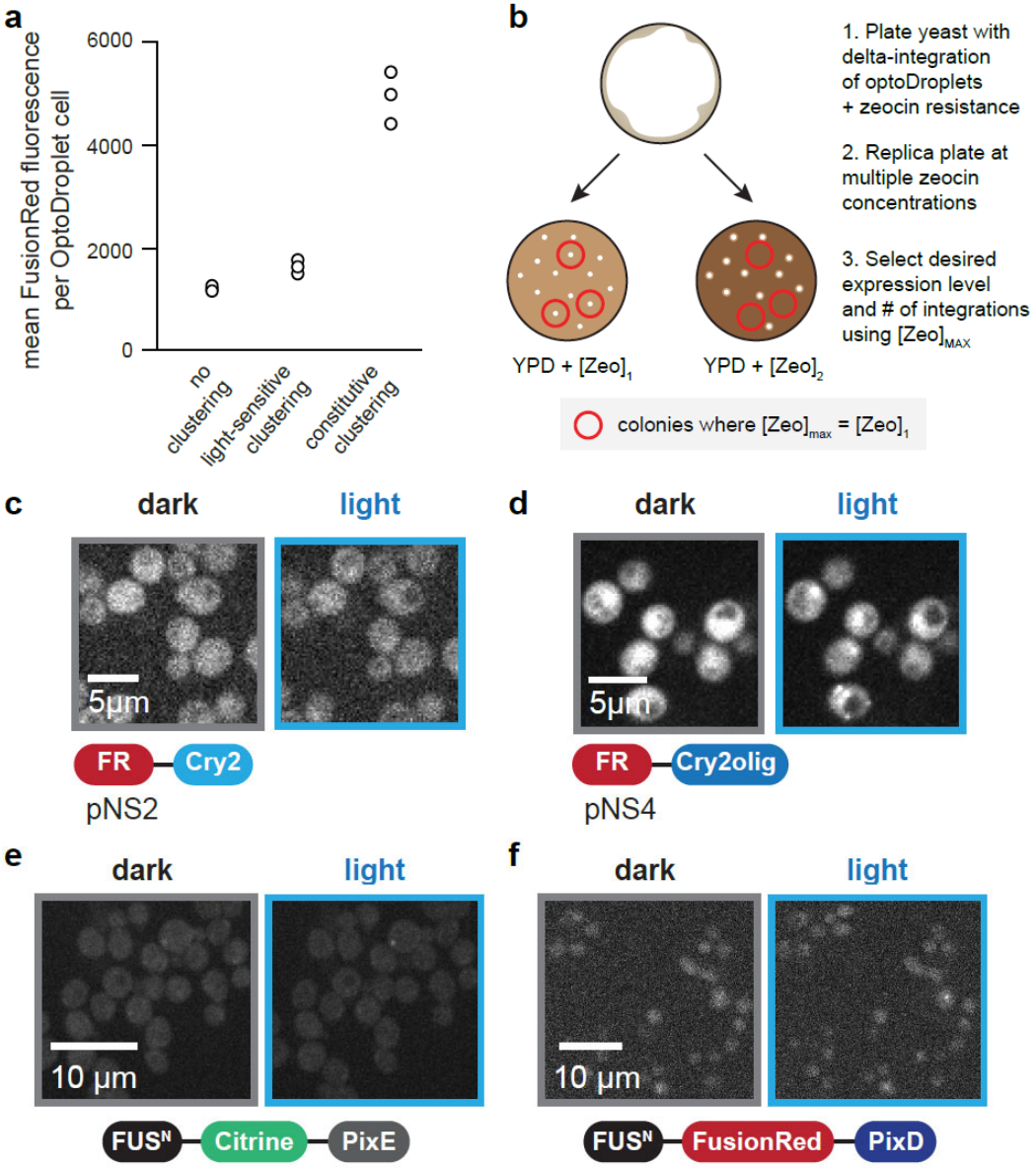
Supplementary Table 2: Yeast Used for this Study

Strain Name	Genotype	Source
BY4741	S288C *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann et al. (main text Ref. 35).
CEN.PK2-1C	*MAT***a** *his3Δ1 leu2*-3_112 *trp1*-289 *ura3*-53	Entian et al. (main text Ref. 37).
YNS21	BY4741 *HIS3*::P_{TEF1}_*VioB*_T_{ACT1}+ P_{PGK1}_*VioA*_T_{CYC1}	This Study
YNS34	YNS21 YARCdelta5::P_{ADH1}_*FUS*^{N}_*Citrine_Cry2Olig_VioC*_T_{ACT1}; YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2Olig_VioE*_T_{ACT1}	This Study
YNS34cterm	YNS21 YARCdelta5::P_{ADH1}_*FUS*^{N}_*Citrine_Cry2Olig_VioC*_T_{ACT1}; YARCdelta5::P_{ADH1}_*VioE_FUS*^{N}_*FusionRed_Cry2Olig*_T_{ACT1}	This Study
YNS34Drop	YNS21 YARCdelta5::P_{ADH1}_*FUS*^{N}_*Citrine_Cry2_VioC*_T_{ACT1}; YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2_VioE*_T_{ACT1}	This Study
YNS36	YNS21 YARCdelta5::P_{ADH1}_*VioC_FUS*^{N}_*Citrine_Cry2Olig*_T_{ACT1}; YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2Olig_VioE*_T_{ACT1}	This Study
YNS36cterm	YNS21 YARCdelta5::P_{ADH1}_*VioC_FUS*^{N}_*Citrine_Cry2Olig*_T_{ACT1}; YARCdelta5::P_{ADH1}_*VioE_FUS*^{N}_*FusionRed_Cry2Olig*_T_{ACT1}	This Study
YNS36Drop	YNS21 YARCdelta5::P_{ADH1}_*VioC_FUS*^{N}_*Citrine_Cry2*_T_{ACT1}; YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2_VioE*_T_{ACT1}	This Study
YNS46	BY4741 *HIS3*::P_{TEF1}_*VioB*_T_{ACT1} + P_{PGK1}_*VioA*_T_{CYC1} + P_{GPD}_*VioE*_T_{ADH1}	This Study
YNS47	CEN.PK2-1C YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2WT*_T_{ACT1}	This Study
YNS47BY	BY4741 YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2WT*_T_{ACT1}	This Study
YNS48	CEN.PK2-1C YARCdelta5::P_{ADH1}_*FusionRed_Cry2WT*_T_{ACT1}	This Study
YNS48BY	BY4741 YARCdelta5::P_{ADH1}_*FusionRed_Cry2WT*_T_{ACT1}	This Study
YNS49	CEN.PK2-1C YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2Olig*_T_{ACT1}	This Study
YNS49BY	BY4741 YARCdelta5::P_{ADH1}_*FUS*^{N}_*FusionRed_Cry2Olig*_T_{ACT1}	This Study
YNS50	CEN.PK2-1C YARCdelta5::P_{ADH1}_*FusionRed_Cry2Olig*_T_{ACT1}	This Study
YNS50BY	BY4741 YARCdelta5::P_{ADH1}_*FusionRed_Cry2Olig*_T_{ACT1}	This Study

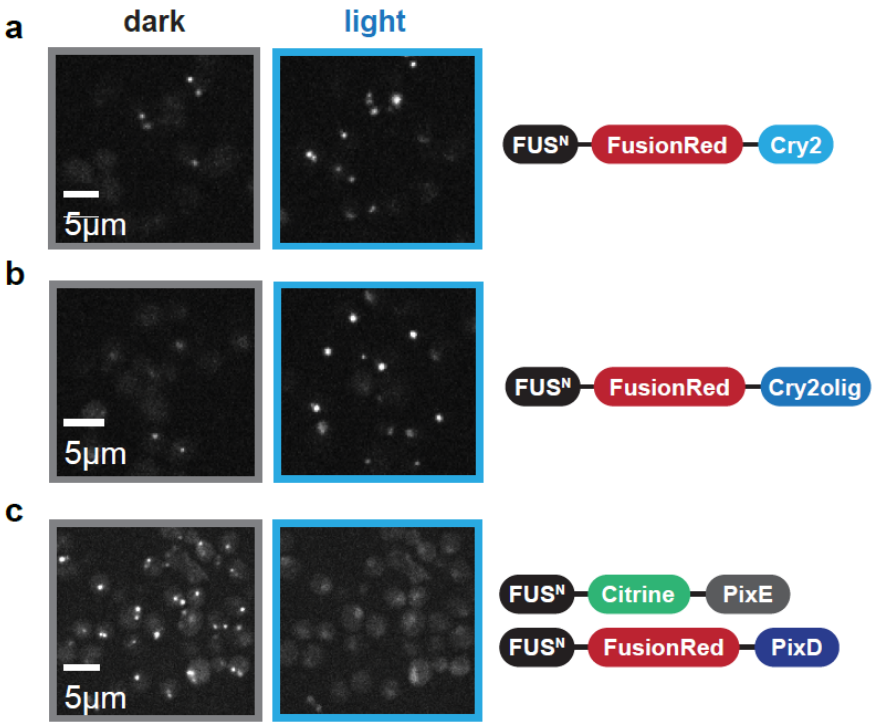
YNS51	YNS46 + pNS7	This Study
YNS52	YNS21 + pNS8	This Study
YNS53	YNS21 + pNS9	This Study
YNS54	YNS52 YARCdelta5::P_{ADH1}_*FUS^N*_*Citrine_Cry2Olig_VioC*_T_{ACT1}	This Study
YNS55	YNS52 YARCdelta5::P_{ADH1}_*VioC_FUS^N_Citrine_Cry2Olig*_T_{ACT1}	This Study
YNS56	YNS53 YARCdelta5::P_{ADH1}_*FUS^N*_*FusionRed_Cry2Olig_VioE*_T_{ACT1}	This Study
YNS57	YNS53 YARCdelta5::P_{ADH1}_*VioE_FUS^N_FusionRed_Cry2Olig*_T_{ACT1}	This Study
YEZ53	CEN.PK2-1C + EZ-L176	This Study
YEZ140	CEN.PK2-1C *HIS3*$_{cg}$	Ref. 1
YEZ231	CEN.PK2-1C *HIS3*::P_{PGK1}_*FUS_Citrine_PixE*_T_{CYC1}	This Study
YEZ231BY	BY4741 *HIS3*::P_{PGK1}_*FUS_Citrine_PixE*_T_{CYC1}	This Study
YEZ232	YEZ231 YARCdelta5:: P_{ADH1}_*FUS^N*_*FusionRed_PixD*_T_{ACT1}	This Study
YEZ232BY	YEZ231BY YARCdelta5:: P_{ADH1}_*FUS^N*_*FusionRed_PixD*_T_{ACT1}	This Study
YEZ234	CEN.PK2-1C YARCdelta5:: P_{ADH1}_*FUS^N*_*FusionRed_PixD*_T_{ACT1}	This Study
YEZ250	YNS21 YARCdelta5::P_{ADH1}_*Citrine_Cry2Olig_VioC*_T_{ACT1}; YARCdelta5::P_{ADH1}_ *FusionRed_Cry2Olig_VioE*_T_{ACT1}	This Study
YEZ255	YEZ282 *HIS3*::P_{PGK1}_*FUS^N*_*Citrine_PixE_VioE*_T_{CYC1}	This Study
YEZ257	YEZ255 YARCdelta5:: P_{ADH1}_*FUS^N*_*FusionRed_PixD_VioC*_T_{ACT1}	This Study
YEZ281	YEZ255 YARCdelta5::P_{ADH1}_*FUS^N*_*FusionRed_PixD*_T_{ACT1}	This Study
YEZ282	BY4741 *LEU2*::P_{TEF1}_*VioB*_T_{ACT1} + P_{PGK1}_*VioA*_T_{CYC1}	This Study
YEZ511	YEZ257 + EZ-L859	This Study
YEZ512	YEZ281 + pNS7	This Study
YEZ553	YEZ255 YARCdelta5::P_{ADH1}_*sFUS_PixD_VioC*_T_{ACT1}	This Study

YEZ554	YEZ255 YARCdelta5::P_{ADH1}_*sFUS_FusionRed_PixD*_T_{ACT1}	This Study
YEZ555	YEZ231 YARCdelta5::P_{ADH1}_*sFUS_FusionRed_PixD*_T_{ACT1}	This Study
MZW342	S288C *MAT***a/α** *HIS3*::P_{Z3}_VioA; *LEU2*::P_{Z3}_VioB; *LYS2*::P_{Z3}_VioE *MET15*::P_{Z3}_VioC; *URA3*::P_{Z3}_VioD; CAN1::P_{ACT1}_yZ_3EV (Ref. 2)	This Study
MZW375	S288C *MAT***a/α** *HIS3*::P_{Z3}_VioA *LEU2*::P_{Z3}_VioB *LYS2*::P_{Z3}_VioE_µNSCCy *MET15*:: P_{Z3}_VioC_ µNSCCy *URA3*::P_{Z3}_VioD CAN1::P_{ACT1}_yZ_3EV (Ref. 2)	This Study
MZW377	S288C *MAT***a/α** *HIS3*::P_{Z3}_VioA *LEU2*::P_{Z3}_VioB *LYS2*::P_{Z3}_VioE_µNSCCy *MET15*:: P_{Z3}_ µNSCCy_VioC *URA3*::P_{Z3}_VioD CAN1::P_{ACT1}_yZ_3EV (Ref. 2)	This Study
MZW378	S288C *MAT***a/α** *HIS3*::P_{Z3}_VioA *LEU2*::P_{Z3}_VioB *LYS2*::P_{Z3}_ELK16_VioE *MET15*:: P_{Z3}_VioC_ ELK16 *URA3*::P_{Z3}_VioD CAN1::P_{ACT1}_yZ_3EV (Ref. 2)	This Study

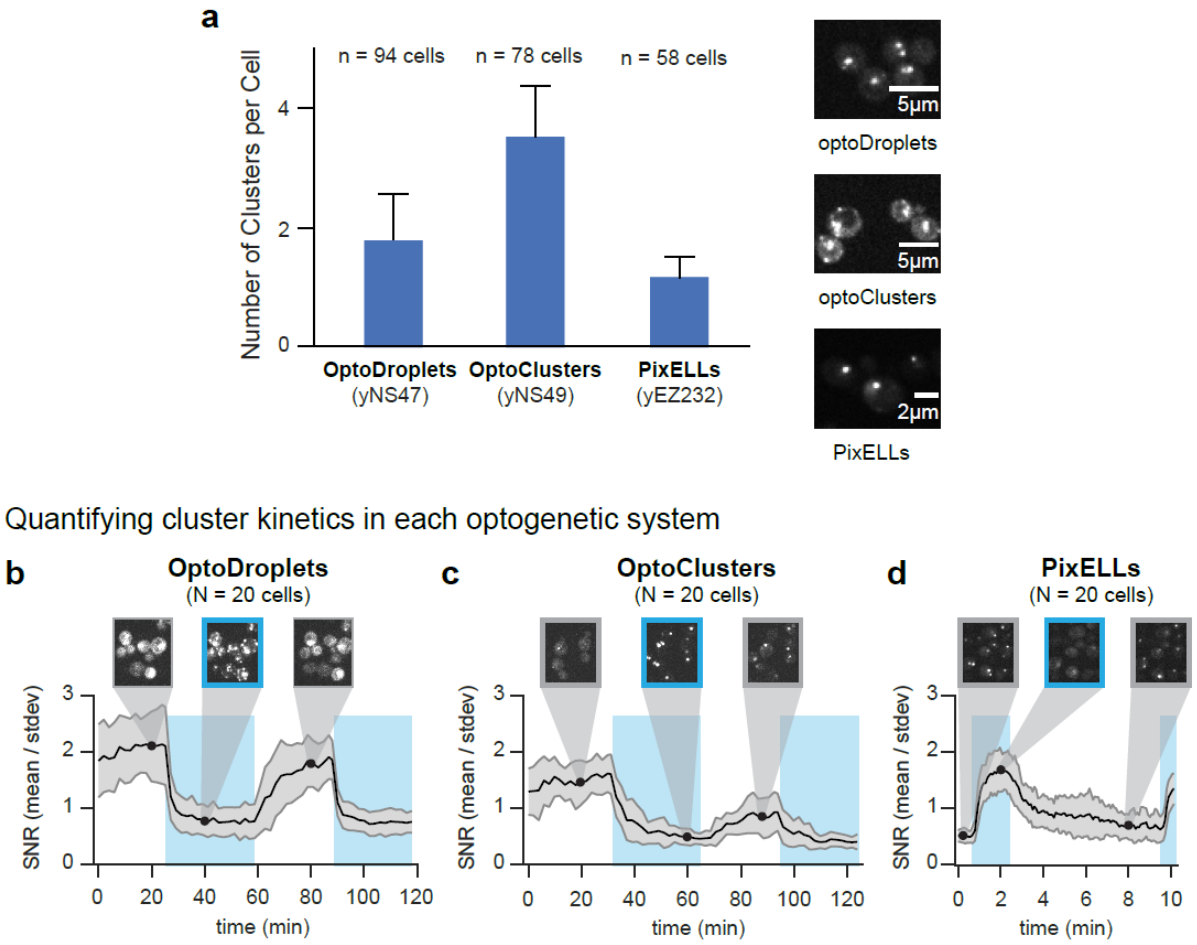
Supplementary Figures and Legends



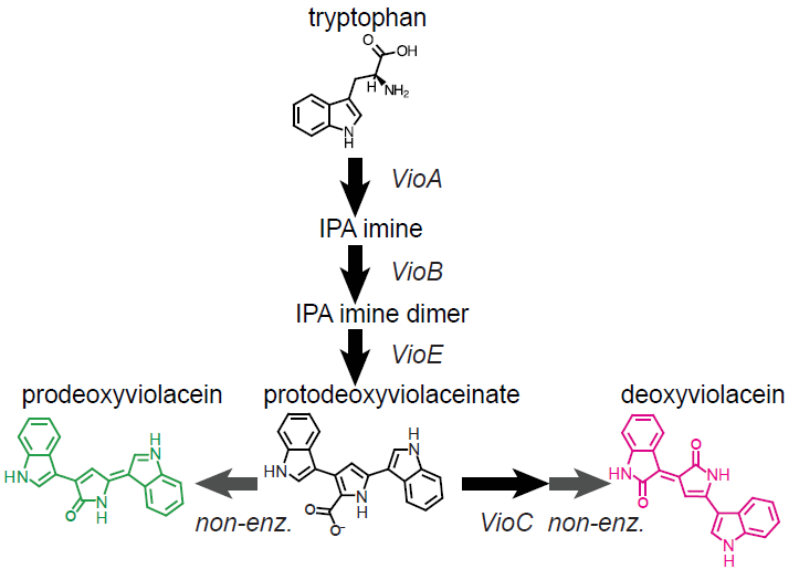
Supplementary Figure 1. Characterizing light-switchable clustering. (**a**) FusionRed expression levels of OptoDroplet cells from Fig. 2c. The mean fluorescence intensity in the FusionRed channel is shown for cells exhibiting different clustering responses. (**b**) Schematic of zeocin selection approach. Yeast with multiple genomic integrations of an optogenetic system are replica-plated onto varying zeocin doses, and relative protein expression is assessed by the highest level of zeocin on which colonies survive (red circles, $[Zeo]_{max}$). (**c, d**) Fluorescent Cry2 and Cry2olig constructs lacking FUS^N tags fail to show clustering in light. Images show FusionRed fluorescence in cells incubated in the dark (left panels) or exposed to 5 minutes of blue light (right panels) in strains YNS48 (top) and YNS50 (bottom). (**e, f**) When expressed alone, FUS^N-Citrine-PixE (shown in **e**) and FUS^N-FusionRed-PixD (shown in **f**) do not cluster.



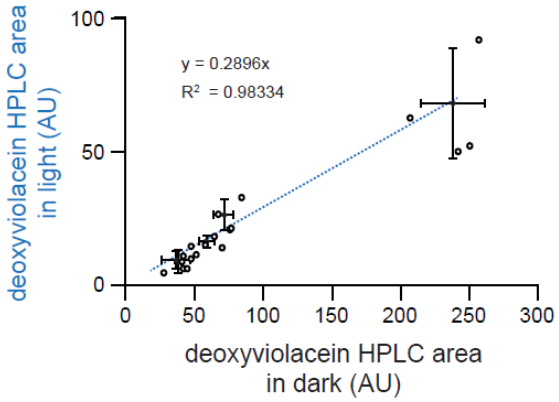
Supplementary Figure 2. Light-dependent synthetic organelle formation in the BY4741 strain of *S. cerevisiae*. (**a**) OptoDroplet formation from dark (left) to 5 minutes of light exposure (right) in YNS47. (**b**) OptoCluster formation from dark (left) to 5 minutes of light exposure (right) in YNS49. (**c**) PixELL dissociation from dark (left) to 1 minute of light (right) in YEZ232BY. For each panel in **a-c** the FusionRed fluorescence images were chosen to be representative of four colonies picked at $[Zeo]_{max}$ = 800 mg/L.



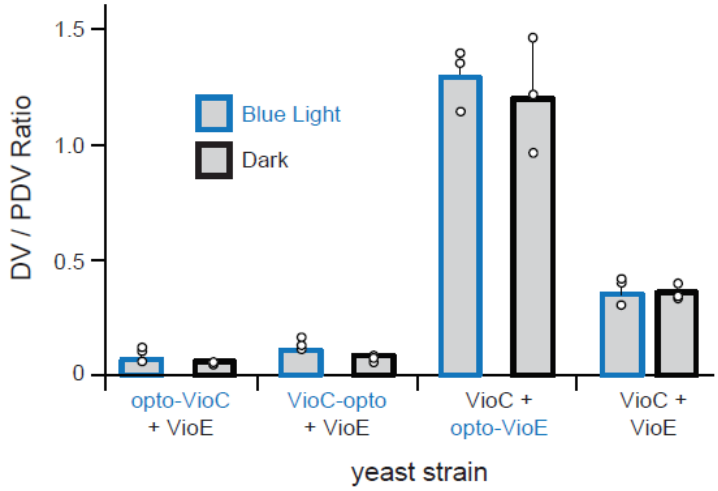
Supplementary Figure 3. Analyses of cluster number and cluster kinetics for the OptoDroplet, OptoCluster and PixELL systems. (**a**) The number of clusters observed after induction with light for 5 minutes (for OptoDroplets and OptoClusters) or in dark-incubated cells (for PixELLs) was quantified from FusionRed fluorescent images. The number of clusters per cell may also vary as a function of expression level and stimulus time. Inset images show representative FusionRed fluorescence images of each of the three strains (YNS47, YNS49, YEZ232) under clustering conditions. (**b-d**) The kinetics of cluster assembly and disassembly for OptoDroplets (YNS47; in **b**), OptoClusters (YNS49; in **c**) and PixELLs (YEZ232; in **d**) during cycles of illumination and dark incubation. The mean intensity and standard deviation of each cell's FusionRed fluorescence was measured to obtain the signal-to-noise ratio: a reproducible, quantitative metric of clustering (y axes). Higher values indicate diffuse localization; lower values indicate clustering. Upper inset images show representative FusionRed fluorescence images of each of the three strains (YNS47, YNS49, YEZ232) at the timepoints indicated.



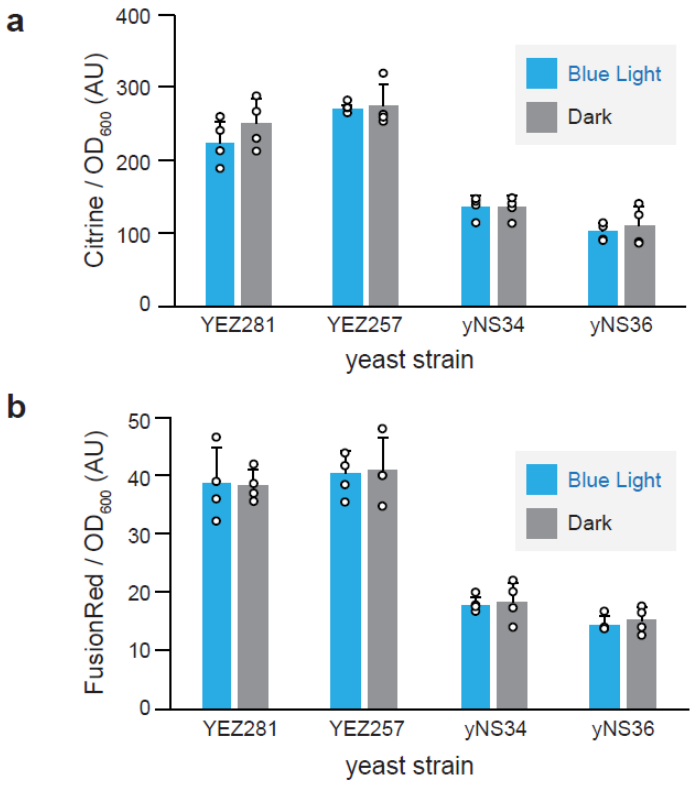
Supplementary Figure 4. The deoxyviolacein synthesis pathway. The production of deoxyviolacein (DV) depends on the relative activities of VioC and VioE. VioE increases production of the protodeoxyviolaceinate (PTDV) intermediate, whereas VioC catalyzes its conversion to DV. High VioC activity would be expected to completely convert PTDV to DV, whereas high VioE activity combined with low VioC activity would be expected to induce loss of PTDV to prodeoxyviolacein (PDV) through non-enzymatic oxidation.



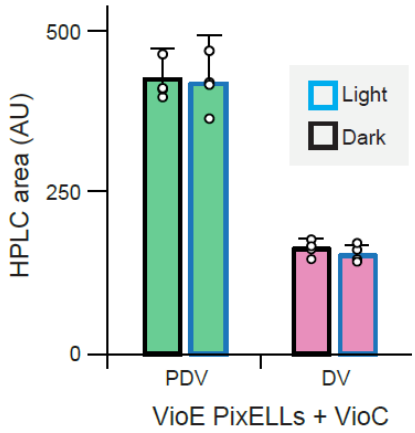
Supplementary Figure 5. Deoxyviolacein degradation curve. Non-optogenetic yeast strains that constitutively produce varying amounts of DV were used to create a standard curve of DV/PDV photo-degradation during fermentation. All strains were subjected to a four-day fermentation that was identical to those performed for the experiments of Fig. 3 and 4, either under blue light or constant darkness. This slope was then used to correct titers for the photo-degradation of DV. Individual points represent YNS51, MZW342, MZW375, MZW377, and MZW378. No differences in growth rate or maximum optical density of these strains in the light and the dark were observed. All data are shown as mean values; error bars represent the standard deviation of four biologically independent 1-ml sample replicates exposed to the same conditions (shown as individual points).



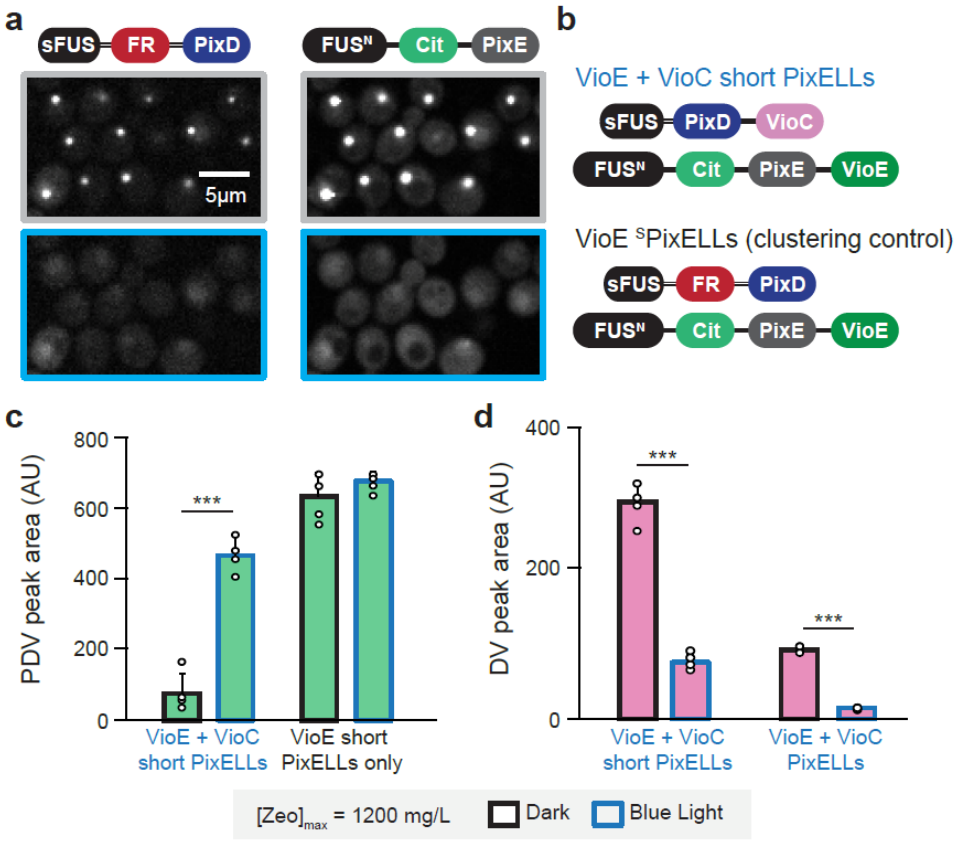
Supplementary Figure 6. DV/PDV ratio for optoCluster-expressing control strains. DV/PDV production ratios of control strains that express VioE and VioC, but are unable to co-localize them into synthetic organelles (strains YNS54, YNS55, YNS56, and YNS51). All strains were subjected to a four-day fermentation that was identical to those performed for the experiments of Fig. 3 and 4 under either blue light or constant darkness. All data are shown as mean values; dots represent individual data points; error bars represent the standard deviation of three biologically independent 1-ml sample replicates exposed to the same conditions.



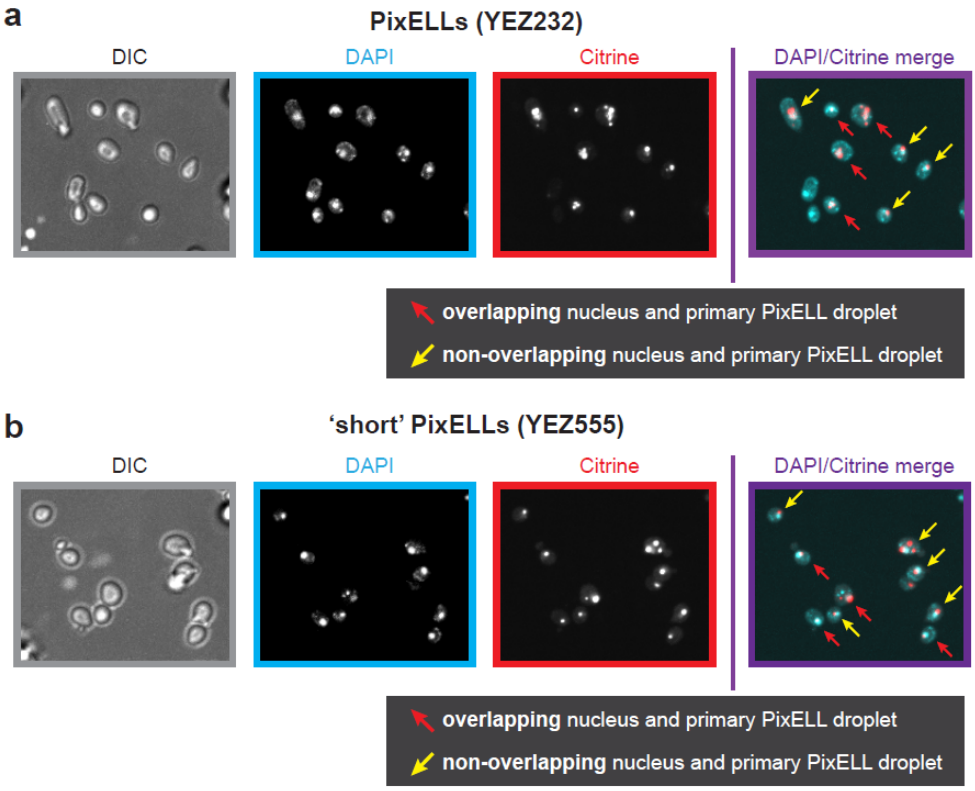
Supplementary Figure 7. Protein concentrations are unaffected by light condition or clustering. Four PixELL and OptoCluster strains harboring various VioE/VioC optogenetic fusions (strains YEZ281, YEZ257, YNS34 and YNS36) were fermented for four days in light or dark conditions as in Figure 3. At the end of the four-day fermentation the Citrine (in **a**) and FusionRed (in **b**) fluorescence levels were measured by plate reader. To account for any differences in cell number, fluorescence measurements were normalized by the optical density at 600 nm (OD_{600}) of the culture. All data are shown as mean values; dots represent individual data points; error bars represent the standard deviation of four biologically independent 1-ml sample replicates exposed to the same conditions.



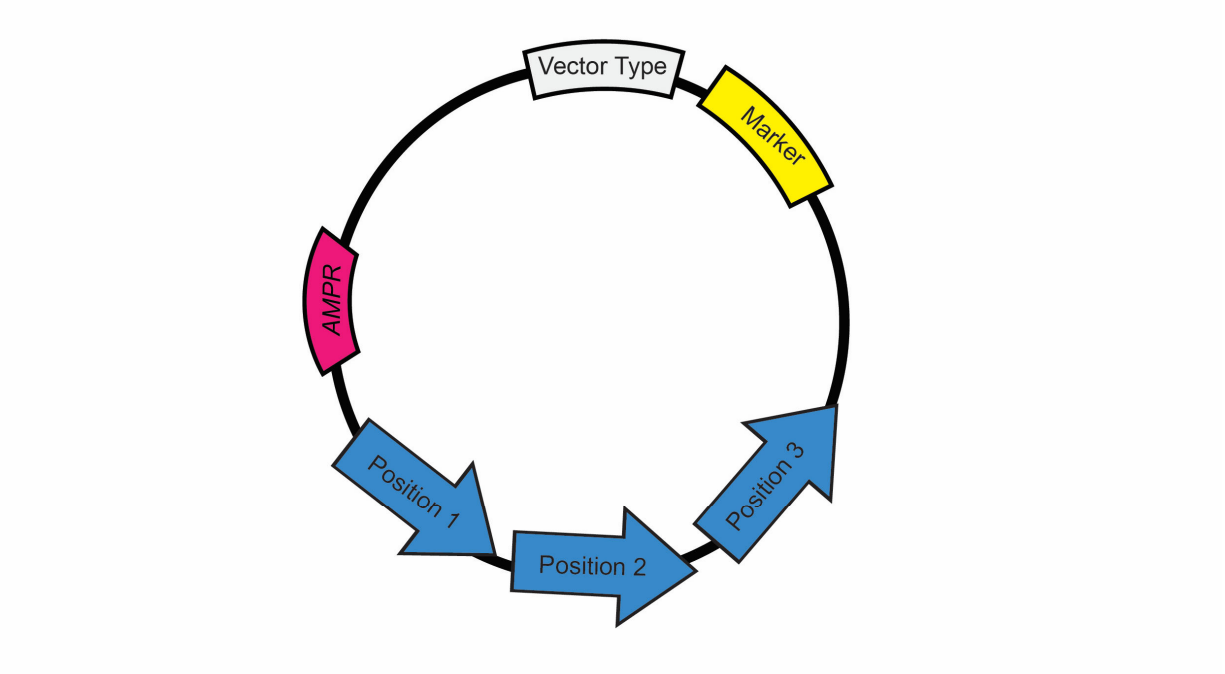
Supplementary Figure 8. Control experiment for PixELL clustering of VioC and VioE. PDV (green bars) and DV (pink bars) titers in a strain expressing VioE-PixELLs and diffuse, un-labeled VioC (YEZ512). All strains were subjected to a four-day fermentation that was identical to those performed for the experiments of Fig. 3 and 4, either under blue light or constant darkness. All data are shown as mean values; dots represent individual data points; error bars represent the standard deviation of four biologically independent 1-mL sample replicates exposed to the same conditions.



Supplementary Figure 9. IDR shortening improves enzyme activity and/or expression. Investigation of PixELLs functionality using constructs with the first 93 amino acids of the FUS^N domain. **(a)** Microscopy images of YEZ555 under different light conditions showing dissociation of the two components of shortened PixELL system. Images are representative of four colonies picked at the conditions specified. **(b)** Constructs tested for dark-inducible metabolic organelles harboring shortened FUS domains (sFUS) on PixELLs-based VioE/VioC co-clusters**. (c)** HPLC quantification of PDV from four day fermentations of YEZ553 (left) and YEZ554 (right, control with no VioC), both of which were selected from $[Zeo]_{max}$ = 1,200 mg/L. Error bars represent standard deviations of 1 mL biological replicates exposed to the same light conditions (n = 4). ***, $p < 0.001$. Statistics are derived using a one-sided t test. **(d)** HPLC quantification of DV from four day fermentations of YEZ553 (left, shortened PixELLs) and YEZ257 (right, Full-length PixELLs), both of which were selected from $[Zeo]_{max}$ = 1,200 mg/L. All data are shown as mean values; dots represent individual data points; error bars represent the standard deviation of four biologically independent 1-ml sample replicates exposed to the same conditions. All experiments were repeated at least three times.



Supplementary Figure 10. Monitoring nuclear vs cytosolic PixELL droplet localization using 4′,6-diamidino-2-phenylindole (DAPI). (**a-b**) PixELL- and short PixELL-expressing yeast strains YEZ232 (in **a**) and YEZ555 (in **b**) were incubated under dark conditions, then fixed and stained with DAPI to compare the localization of PixeLL droplets and DNA (see Methods). Still images show representative cells under DIC imaging (gray border), after maximum intensity projections in the DAPI channel (blue border), after maximum intensity projections in the Citrine channel (green border), and as a merged DAPI/Citrine image (purple border). Cells are marked to indicate whether they harbor cytosolic PixELL clusters that are distinct from the DAPI-stained nucleus (yellow arrows), or whether they harbor PixELL clusters that closely overlap the DAPI-stained nucleus (red arrows).



Supplementary Figure 11. General scheme of vectors. Vector map shows the relative orientation of the three positions listed in Supplementary Table 1, in which different genes (including promoters and terminators) were assembled, using a previously described multiple gene insertion strategy[3]. All vectors have an ampicillin resistance marker (*AMPR*) for cloning in *E. coli* and a selection marker for *S. cerevisiae* (Marker). Vector types include CEN/ARS, 2μ, or integrative[3-6].

Supplementary Note 1: Optogenetic system gene sequences used in this study

Highlighted in cyan/blue: the FUS intrinsically-disordered region (FUS^N)

Highlighted in green: fluorescent protein domains

Highlighted in red: optogenetic system protein domains

FUS^N-FusionRed-Cry2

ATGGCTAGCgcctcaaacGATTATACCCAACAAGCaacccAAAGCTATGGGGCCTACCCCACCCAGCCCG
GGCAGGGCTATTCCCAGCAGAGCAGTCAGCCCTACGGACAGCAGAGTTACAGTGGTTATAGCCAGTCCAC
GGACACTTCAGGCTATGGCCAGAGCAGCTATTCTTCTTATGGCCAGAGCCAGAACACAGGCTATGGAACT
CAGTCAACTCCCCAGGGATATGGCTCGACTGGCGGCTATGGCAGTAGCCAGAGCTCCCAATCGTCTTACG
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FUS^{N}-FusionRed-Cry2olig

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FUS^{N}-Citrine-PixE

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FUS^{N}-FusionRed-PixD

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Supplementary Video Legends

Supplementary Video 1. OptoDroplet formation and dissociation in *S. cerevisiae.*

Time lapse imaging of the optoDroplet yeast strain YNS47, expressing delta-integrated copies of FUS^N-FusionRed-Cry2 selected from $[Zeo]_{max}$ = 800 mg/L. Cells show repeated reversible light-dependent formation and dissociation of clusters. Images were acquired with RFP imaging settings (561 nm excitation) every 10 sec for 28 min. Blue box in movie signifies timing of light delivery. Scale bar indicates 10 μm.

Supplementary Video 2. OptoCluster formation and dissociation in *S. cerevisiae.*

Time lapse imaging of the optoCluster yeast strain YNS49, expressing delta-integrated copies of FUS^N-FusionRed-Cry2olig selected from $[Zeo]_{max}$ = 800 mg/L. Cells show reversible light-dependent formation and dissociation of clusters, with some clusters persisting in un-illuminated conditions. Images were acquired with RFP imaging settings (561 nm excitation) every 10 sec during initial illumination phase, every 1 min during dark reversion phase, and every 10 sec during the second illumination phase. Note that Cry2olig exhibits a longer lit-state lifetime (~20 min) than Cry2 (~2 min), necessitating a longer incubation to capture dark-state reversion. Blue box in movie signifies timing of light delivery. Scale bar indicates 10 μm.

Supplementary Video 3. PixELL formation and dissociation in *S. cerevisiae.*

Time lapse imaging of the optoCluster yeast strain YEZ232, expressing delta-integrated copies of FUS^N-FusionRed-PixD selected from $[Zeo]_{max}$ = 1,200 mg/L and a single copy of FUS^N-Citrine-PixE. Cells show reversible light-dependent formation and dissociation of clusters. Images were acquired with RFP imaging settings (561 nm excitation) every 10 sec. Blue box in movie signifies timing of light delivery. Scale bar indicates 10 μm.

Supplementary References

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