SUPPLEMENTARY MATERIAL

Engineered BRET-Based Biologic Light Sources Enable Spatio-Temporal Control Over Diverse Optogenetic Systems

Kshitij Parag-Sharma[1,14], Colin P. O'Banion[2,3,14], Erin C. Henry[4,5], Adele M. Musicant[6], John L. Cleveland[7], David S. Lawrence[2,8,9,10], and Antonio L. Amelio[11,12,13]*

[1]Graduate Curriculum in Cell Biology and Physiology, Biological and Biomedical Sciences Program, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[2]Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA
[3]Neuronal Signal Transduction, Max Planck Florida Institute for Neuroscience, Jupiter, FL 33458, USA
[4]Lineberger Comprehensive Cancer Center, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[5]Division of Oral and Craniofacial Health Sciences, UNC Adams School of Dentistry, University of North Carolina, Chapel Hill, NC 27599, USA
[6]Graduate Curriculum in Genetics and Molecular Biology, Biological and Biomedical Sciences Graduate Program, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[7]Department of Tumor Biology, Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA
[8]Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599, USA
[9]Department of Pharmacology, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[10]Molecular Therapeutics Program, Lineberger Comprehensive Cancer Center, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[11]Department of Cell Biology and Physiology, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[12]Cancer Cell Biology Program, Lineberger Comprehensive Cancer Center, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[13]Biomedical Research Imaging Center, UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[14]Co-first author

***Correspondence:** antonio_amelio@unc.edu

CONTACT FOR REAGENTS AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Antonio L. Amelio (antonio_amelio@unc.edu).

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MATERIALS AND METHODS

Plasmid construction

CeNLuc: Cloning of the monomeric Cerulean (mCerulean3, an enhanced CFP variant)[1] fusion to NanoLuc fusion (CeNLuc) was similar to that of the previously described GpNLuc LumiFluor[2] (Addgene #70185, Figure S5). Briefly, the mCerulean3 cDNA lacking a stop codon was PCR amplified and cloned with 5' *BspMI* and 3' *EcoRV* restriction enzyme sites into the pRetroX-Tight-Puro vector (Clontech) under the control of the *PGK* promoter and in place of the *Puromycin*^{R} cassette. In-frame fusion of an DISGG peptide linker and NanoLuc cDNA to mCerulean3 was achieved by a restriction enzyme-free, two step PCR cloning protocol using two separate sets of PCR primers designed with overlapping regions of homology to the new pRetroX-mCerulean3 vector and NanoLuc. These were then used to amplify each respective region and transformed into competent *E. coli*. Recombination of the DISGG peptide linker-NanoLuc fragment into the pRetroX-mCerulean3 vector was confirmed by sequencing. The CeNLuc LumiFluor fusion was then PCR amplified and cloned with 5' *PmeI* and 3' *NotI* restriction enzyme sites into the pRRL lentiviral vector (kindly provided by J. Zuber, Research Institute of Molecular Pathology, Vienna, Austria) under the control of the *EF-1α* promoter and confirmed by sequencing. The pRP-8xgRNA-minCMV Promoter-FLuc *firefly* luciferase (FLuc) reporter construct was custom synthesized (Cyagen Bioscience) and contains eight repeats of the sequence 5′-AAAGGTCGAGAAACTGCAAA-3′ upstream of a minimal *CMV* promoter and the gene encoding FLuc. Annotated sequences and a map for the promoter and FLuc transgene are shown in Figure S6.

Mito-CeNLuc: The mitochondrial membrane targeted CeNLuc (OMMCeNLuc) was generated by subcloning a 5' *KpnI* and 3' *BamHI* tagged CeNLuc fragment PCR amplified from pRetroX-CeNLuc, digesting the fragment with each respective restriction enzyme, and then ligating the fragment into the previously described pEF1α-T2-mCherry-bPAC2 plasmid.[3]

Nuc-CeNLuc: The nuclear targeted CeNLuc (NLS-CeNLuc) was generated by digesting the pEF1α-T2-mCherry-OMMCeNLuc plasmid with *EcoRI* and *KpnI* to excise the T2 cassette and cloning a synthetic fragment assembled from annealed oligos containing tandem 2x SV40 NLS sites tagged with 5' *EcoRI* and 3' *KpnI* sites.

iRFP670-micro: A pLL7.0 backbone was linearized via restriction digest with *AgeI* and *BamHI* (NEB). A Gblock (IDT) encoding SspB R73Q ("micro")[4] and a 3' homology region with pLL7.0 including the *BamHI* restriction site was purchased. iRFP670[5] was amplified by PCR using primers that appended a pLL7.0 homology region including the *AgeI* restriction site to the 5' end of the amplicon and a micro homology region to the 3' end of the amplicon. Gene fragments and pLL7.0 backbone were assembled via Gibson assembly. In brief, inserts and backbone were used at a 3:3:1 molar ratio and assembled using 2X Gibson master mix solution (NEB). Assembly was performed by incubation at 50 °C for 30 min followed by transformation into Stbl3 (ThermoFisher) chemically competent cells and plating onto selective LB-agar. Colonies were picked and screened via restriction digest with ClaI (NEB). Positive colonies were picked and verified via Sanger sequencing.

Additional plasmids: Venus-iLID-Mito (Addgene #60413) was a gift from Brian Khulman, CRY2-CreN (Addgene #26888)[6] and CIBN-CreC (Addgene #26889)[6] were gifts from Chandra Tucker, *loxP*-STOP-*loxP*-ZsGreen (Addgene #51269)[7] was a gift from Pawel Pelczar, *loxP*-STOP-*loxP*-FLuc (Addgene #60622)[8], dCas9-VP64 (Addgene #47107)[9], CibN-dCas9-CibN (Addgene #60553)[9], Cry2FL-VP64 (Addgene #60554)[9], gRNA-eGFP (Addgene #60719)[9], and the pGL3-Basic-8x-gRNA-eGFP reporter (Addgene #60718)[10] were gifts from Charles Gersbach, FKF1-VP16 (Addgene #42499)[11] and GI-Gal4 DBD (Addgene #42500)[11] were gifts from Ricardo Dolmetsch, and the 5xGAL4::UAS-FLuc reporter was previously described.[12]

Protein production and characterization

Protein production: Briefly, a vector containing Nluc or GpNluc with a C-terminal 6X His tag was transformed into BL21(DE3) Rosetta 2 chemically competent cells (Novagen) and plated on LB-agar supplemented with 200 µg/mL ampicillin and 34 µg/mL chloramphenicol. The following day, single colonies were picked and used to inoculate 10 ml starter cultures in selective LB. Starter cultures were grown overnight and added to 1 L of selective LB the following morning. Cultures were grown at 37 °C until they reached an OD600 of 0.4 - 0.8. Expression was then induced by the addition of IPTG (Acros Organics) to a final concentration of 1 mM. The culture temperature was reduced to 20 °C and expression carried out for 18 hr. Cultures were centrifuged the following day and pellets were stored at -80 °C until purification.

Protein purification: Purification was performed via IMAC utilizing Ni-NTA resin (Pierce). Cells were lysed in 50 mM sodium phosphate pH 7.4 with 300 mM NaCl with HALT protease inhibitor (Pierce) and 100 µl per 50 ml DNAse (Sigma). Lysis was performed via 5x 1 min sonication pulses with a 1 min cooling period between each round of sonication. Lysates were clarified via centrifugation at 17,000 x *g* for 15 min. Clarified lysates were loaded into a 50 mL superloop for purification via FPLC (GE AKTA Prime Plus) and purification was performed. Fractions were collected and concentrated via centrifugation through a 10 kDa MWCO filter (EMD Millipore). Each protein was further purified via anion exchange chromatography through a HiPrep Q 16/10 column (GE Life Sciences) and purified fractions were concentrated via centrifugation through a 10 kDa MWCO filter. Purified proteins were stored at -20 °C in Tris pH 7.5 150 mM NaCl and 50% Glycerol (Sigma).

Protein characterization in vitro: For power density measurements, purified proteins were serially diluted in 1x DBPS (Gibco) and 100 µL of 2x protein dilution was added per well of a 96 well opaque white flat bottom plate (Costar). Luminescence was initiated by adding 100 µL of 200 µM furimazine (Promega); final in well concentration of 100 µM. Immediately post addition

of furimazine, the protein/furimazine mix was rapidly pipetted up and down three times to ensure complete mixing and light power output was measured using a Fiber Optic Power Meter (THOR labs cat. #: PM20A) by inverting the power meter onto the light emitting well. The highest power reading observed within the first 5 s was noted. Experiment was performed in a dark room to minimize ambient light contamination. Incident wavelength was set to 510 nm (Em_{max} of GpNLuc).

Protein characterization in cellulo: Emission spectral scans of native lysates from HEK293T cells expressing NanoLuc, GpNLuc, or CeNLuc were performed in white opaque 96-well microplates (OptiPlate-96, PerkinElmer Inc.) using a Cytation5™ microplate reader (BioTek Instruments, Inc.). Emission spectra were recorded from 400 nm to 600 nm using the integration time of 1000 ms with 5 nm step increments. The assay reagent for the emission spectral scans contained 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, cOmplete™ EDTA-free protease inhibitors (Roche Diagnostics) and 50 µM FZ (Promega).

Cell culture

HeLa cells (ATCC CCL-2) were maintained in DMEM (Gibco) + 10% FBS (Gibco) + 1% GlutaMAX-I (Gibco) + 1X Pen/Strep (Gibco). HEK293T cells (ATCC; CRL-11268) were maintained in DMEM medium (Invitrogen) supplemented with 10% FBS (Premium Select; Atlanta Biologicals), GlutaMAX (Invitrogen), and PSG (penicillin, streptomycin, and L-glutamine, Invitrogen) and passaged using TrypLE™ purified, recombinant cell-dissociation enzymes (ThermoFisher Scientific). Cells procured from ATCC were characterized by Short Tandem Repeat (STR) profiling.

LumiFluor stable cell line generation and characterization

CeNLuc stable lines were generated using established viral transduction protocols. HEK293T cells stably expressing Lenti:CeNLuc were selected using 0.5 µg/mL Puromycin and HEK293A

cells stably expressing Retro:CeNLuc were FACS sorted. Cell purity (% cells positive for CeNLuc) was monitored weekly, fresh cells were thawed after 15 passages in culture.

CeNLuc stable cell line photon output (IVIS kinetic): HEK293T and HEK293A cells stably expressing the Lenti/Retro CeNLuc, respectively, were trypsinized and resuspended in Imaging media (DMEM + 1xPSG + 1xGlutaMAX + 1xHEPES). Cell densities were counted using an automated cell counter (BioRad cat. #: TC20) and diluted to a density of 1 million/mL. Cells were then serially diluted to the desired densities using the Imaging media. 100 µL/well of cells were added to an opaque white 96 well plate (Corning cat. #: 3917). 100 µL of 2x furimazine diluted in Imaging media was added per well, final in well concentration of 20 µM, 1.25 µM, or 0.625 µM was used. Plates were incubated at room temperature for 5 min to allow the signal to equilibrate. Total photon output was measured using the IVIS Kinetic (PerkinElmer). A 50 ms exposure/f stop=1 was used for the Lenti::CeNLuc stable cells and an exposure of 2 s/f stop=2 was used for the Retro::CeNLuc stables. For the direct Retro versus Lenti stable comparison, an exposure of 50 ms/f stop=2 was used.

Stable cell line power output (THOR power meter): HEK293A cells stably expressing the Retro::CeNLuc were trypsinized and resuspended in Imaging media (DMEM + 1xPSG + 1xGlutaMAX + 1xHEPES). Cell densities were counted using an automated cell counter (BioRad cat. #: TC20) and diluted to a density of 2.5 million/mL. Cells were then serially diluted to the desired densities using the Imaging media. 200 µL/well of cells were added to an opaque white 96 well plate (Corning cat. #: 3917). 50 µL of furimazine, diluted in Imaging media, was added per well to a final concentration of 20 µM. Cells + furimazine mix was rapidly pipetted up and down three times to ensure complete mixing and light power output was measured using a Fiber Optic Power Meter (THOR labs cat. #: PM20A) by inverting the power meter onto the light emitting well. The highest power reading observed within the first 5 s was noted. Experiment was performed in a dark room to minimize ambient light contamination. Incident wavelength was set to 474 nm for CeNLuc.

CeNLuc copy number qPCR: RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel, #740955) according to the manufacturer's instructions. RNA was eluted in RNase-free water and quantified using a BioTek Cytation 5 plate multi-mode reader spectrophotometer. cDNA was synthesized using the iScript cDNA Synthesis Kit (BioRad #170-8891). Quantitative RT-PCR (qRT-PCR) was performed using FastStart Universal SYBR Green Master (Roche #04913850001), CeNLuc primers used were as follows, *sense* 5'-AACGAGAAGCGCGATCACAT-3' and *antisense*- 5'- CGAGTGTGAAGACCATACCTCC-3'.

Transfection and luciferase assays

HEK293T: Cells were counted (TC20™ Automated Cell Counter, BioRad) and $1x10^5$ live cells/well plated into 24-well plates (Falcon) and transfected 16 - 24 hr later using Lipofectamine® 3000 (ThermoFisher Scientific) according to the manufacturer's instructions (a 1:3 ratio for DNA mass:Lipo300 vol was used). For the FKF1:GI experiments, each well was transfected with 500, 250, 500, 250 ng of LumiFluor, 5xGAL4::UAS-FLuc, GI-Gal4 DBD, and empty vector or FKF1-VP16 plasmids, respectively. For the CRY2:CIBN experiments using the dCas9 system, each well was transfected with 500, 200, 300, 300, 200 ng of LumiFluor, 8xgRNA-minCMV-FLuc, gRNAs (gifts from Charles Gersbach)[10], CIBN-dCas9-CIBN, and empty vector or CRY2-VP64 plasmids, respectively. For the CRY2:CIBN experiments using the split Cre recombinase system, each well was transfected with 500, 250, 500, 250 ng of LumiFluor, *loxP*-STOP-*loxP*-FLuc, CIBN-CreC, and empty vector or CRY2-CreN plasmids, respectively. For the split Cre recombinase experiments using the pMagnet system, each well was transfected with 450, 500, 50, ng of *loxP*-STOP-*loxP*-FLuc and Cyto or Nuc CeNLuc and pMagnet or empty plasmids, respectively. Cells were illuminated by FZ substrate administration as indicated and activation of *firefly* luciferase reporter expression was measured on a Cytation5™ microplate reader (BioTek Instruments, Inc.) following cell lysis and addition of Brite-Glo™ Luciferase Assay Substrate (Promega).

HeLa: Cells (ATCC CCL-2) were plated at 1.5×10^5 cells per plate in 35 mm glass bottom

dishes (Mattek) for all experiments except for the pulsatile recruitment experiments. For pulsatile recruitment experiments, 10^3 cells were plated into ibidi μslide VI0.4 perfusion chamber slides. Transfections were performed using Polyplus JetPrime transfection reagent, according to the manufacturers protocol (a 1:3 ratio of DNA mass:JetPrime vol was used). Prior to adding DNA:JetPrime complexes to the cells, complete media was exchanged for Opti-mem (Gibco) and transfection cocktails were added directly to cells. Cells were incubated with transfection cocktails for 3 hr before media was exchanged for complete media. Cells were incubated overnight in complete media before imaging experiments were performed. For the iLID experiments, cells were transfected with a ratio of 2:1:1 for LumiFluor:iRFP670micro:Venus-iLID-Mito by mass was used.

Microscopy and image analysis

HEK293T cells: Static images were acquired with an Olympus IX73 fluorescence microscope (10x and 20x magnification) using a DP80 color/monochrome cooled CCD camera and cellSens™ software v1.11 (Olympus).

HeLa cells/iLID system: All microscopy was performed on inverted Olympus IX81 microscope equipped with a Hamamatsu ORCA-Flash4.0 camera, 40x oil immersion Plan S-Apo NA 1.3 objective and CFP, FITC, TxRed, and Cy5 filter cubes from Semrock. All imaging was performed with 2 x 2 binning and all cells were incubated in L-15 media during imaging experiments. Images were collected at indicated intervals. Light stimulation of iLID was induced by 100 ms pulse of light using the FITC filter cube (~ 488 nm). For all experiments except pulsatile furimazine application, indicated concentrations of furimazine were added at indicated time as 10 X stock solutions in L-15. For pulsatile experiments, cells were perfused with L-15 for 2 min followed by perfusion with 10 μM furimazine in L-15 for 2 min. furimazine was washed out by perfusion with L-15 for 2 min and imaging was continued for another 8 min after washout. Application of furimazine and washout was repeated a total of 3 times.

iLID experiment image analysis: Each image stack was registered and aligned using FIJI's (ImageJ) SIFT registration plugin with default values. For each cell measured, 6 ROIs were defined: 3 mitochondrial and 3 adjacent cytoplasmic regions as determined by Venus-iLID-Mito images. In addition, 3 large background measurements were taken in regions with no fluorescence. All measurements were mean fluorescent intensity. The average background per frame was subtracted from each ROI. The ratio of Mito:Cyto association was determined by dividing the mean fluorescent intensity of a mitochondrial ROI by the mean fluorescent intensity of its adjacent cytoplasmic ROI. Ratios were normalized to t = 0 min by division (Vt = Rt/R0 where Rt = Mito:Cyto ratio at time t and R0 = initial Mito:Cyto ratio) yielding the relative change in mitochondrial association over time. All ROIs were averaged and plotted as a scatter plot with SEM.

Live cell imaging perfusion system

To perfuse and wash away FZ, we built a gravity driven perfusion system (Figure S4). All supplies were acquired from Warner Instruments except for the chamber slides and slide luer connectors which were purchased from ibidi. Briefly, 10 mL luer lock syringes were attached to PE-160 tubing via luer stopcocks. The tubing from the stopcocks ran into flow valves which fed into PE-160 tubing that led to a 2 channel to 1 perfusion manifold. The perfusion manifolds mixed buffer and fed into more PE-160 tubing which was connected by luer connection to the luer connector which led to the imaging chamber (μslide VI0.4). The effluent traveled through an exit luer connector and into a 50 mL waste collection tube.

Furimazine

All furimazine used throughout this study was purchased from Promega (cat. #: N1110). This stock (~ 5mM) was diluted using 1x DPBS or appropriate cell culture media as indicated. Fresh stocks were made immediately prior to use to minimize the possibility of furimazine precipitating out of the solution.

Statistical analysis

Data reflect mean ± SD or mean ± SEM as indicated, and biological and technical replicates are indicated in the figure legends. Statistical significance (*P* value) was determined by comparing samples within the FZ substrate administered condition ("Light") using Student's *t*-test (Prism v6.0c, GraphPad). Qualitative images shown are representative of replicates from a minimum of two independent experiments.

iLID system: Time to maximal association was determined by averaging the difference between the time of FZ addition and time at which maximal mitochondrial association occurred. Fold accumulation was determined by averaging the maximal fold change for each data point. Half-life was determined via fitting a one-phase decay function to data starting at the maximal accumulation point.

SUPPLEMENTARY FIGURES

Figure S1. Characterization of Power and Photon Output for the Cyan-tuned LumiFluor Biologic Light Source (CeNLuc), Related to Figure 1.

(A) Schematic of the pRetroX retrovirus and pRRL lentivirus used to express CeNLuc via the *PGK* or *EF1α* promoters, respectively.

(B) Representative images of HEK293T cells transfected with the cyan (*left, CeNLuc*) or the green (*right, GpNLuc*) LumiFluor BRET molecules. Merged brightfield and fluorescence images are shown.

(C) Quantification of *CeNLuc* mRNA levels in cells transiently transfected versus cells transduced and stably integrated with each respective construct (n = 2 - 4 ± SEM).

(D-E) Comparison of total luminescent light output in HEK293 cells stably expressing either the lentiviral (D) or retroviral (E) CeNLuc expression constructs following administration of the indicated doses of FZ (n = 3 ± SEM). Luminescence intensity was measured using a BioTek Cytation 5 plate reader, exposure = 20 ms at a gain of 100.

(F-H) Total photon output [p/s/cm^2/sr] from 50, 100, 500, 1000, 5000, 10000, 25000, 50000 or 100,000 HEK293 cells stably expressing the lentiviral and retroviral expression constructs was quantified using an IVIS Kinetic bioluminescence imager. (F) Photon output directly comparing retroviral versus lentiviral CeNLuc stable cells administered 20 μM FZ was captured using an exposure= 50 ms/f stop= 2. Additionally, the photon output across multiple doses of FZ (0.625, 1.25 or 20 μM FZ) was quantified for lentiviral (G; exposure= 50 ms/f stop= 1) or retroviral construct (H; exposure= 2s/f stop= 2) expressed CeNLuc (n = 3). Data in F-H represent mean ± SEM, n = biologic replicates and all experiments were performed with at least 2 - 4 technical replicates per condition.

(I-K) Total power output of the HEK293A cells stably expressing the retroviral expressed CeNLuc was quantified using a fiber optic power meter (THOR labs, PM20A). (I) *Inset*, representative

image of the HEK293A retroviral CeNLuc stable cells seeded at increasing densities generating blue light upon addition of 20 μM FZ (50,000, 100,000, 200,000, 300,000, 400,000 or 500,000 cells/well were used). The correlation between total optical power output and the number of cells expressing CeNLuc is linear (FZ = 20 μM, n = 3 ± SEM, R^2 = 0.9704). (J) Raw power output data from (I) was used to calculate the optical power densities generated by CeNLuc expressing cells. (K) Raw power output data from (I) was normalized to the number of cells used per condition in order to generate total power output per individual cell. Each cell stably expressing the retroviral CeNLuc expressing construct was found to generate ~450 fW of power. Per cell power output is independent of the number of cells used (R^2 = 0.0263).

Figure S2. Illustration of Light-Activated Transcriptional Reporter Systems and Optimization of Experimental Parameters. Related to Figure 2.

(A-C) Schematic of light-sensitive transcription systems whereby the photosensitive protein and interaction partner are physically separated (*left*) until cell autonomous biologic light is generated by the CeNLuc LumiFluor following administration of FZ (*right*). (A) The light-activated split Cre recombinase system employs the CRY2-CIBN dimerization partners to reconstitute a functional Cre recombinase molecule capable of inducing *loxP* recombination and excision of the STOP cassette upstream of a *firefly* luciferase reporter. (B) The Gal4-DBD:VP16-AD transcription factor complex is assembled upon light-induced FKF1-VP16 interaction with DNA-bound Gal4-GI at 4xGal4::UAS promoter sequences upstream of a *firefly* luciferase reporter (*left*). To optimize CeNLuc-mediated activation, HEK293T cells were transfected with 500 ng of the pRetroX-CeNLuc construct at the indicated ratios of the FKF1-VP16 and GI-Gal4 fusions (*right*). (C) The gRNA-directed dCas9:VP64-AD transcription factor complex is assembled upon light-induced CRY2-VP64 interaction with CIBN-dCas9-CIBN at gRNAs localized to tandem 8xgRNA promoter sequences upstream of a *firefly* luciferase reporter (*left*). Optimization was performed in HEK293T cells transfected with 500 ng of the pRetroX-CeNLuc construct and varying ratios of the gRNA,

CRY2-VP64, CIBN-dCas9-CIBN, or 8x gRNA luciferase reporter (*right*). For all systems tested, CeNLuc-activated transcription is detected with a *firefly* luciferase reporter before ("Dark") and after ("Light") illumination induced by addition of 20 μM FZ. Data are expressed as mean ± SEM, n = biologic replicates. All experiments were performed with at least 3 - 4 technical replicates per condition.

Figure S3. Dynamic Biologic Light Regulation Triggers tagRFP-micro Association with iLID-mito on Mitochondria, Related to Figure 3.

(A) Representative images of fixed HeLa cells that are co-transfected with tagRFP-micro and the mitochondrial-targeted iLID-mito system plus either a diffuse, cytoplasmically expressed CeNLuc or an outer mitochondrial membrane targeted CeNLuc (OMMCeNLuc). Addition of FZ (20 μM), and activation of the cyan (CeNLuc) light source therein, leads to rapid recruitment of the tagRFP-micro to the mitochondria. Mitochondrial recruitment was quantified by evaluating its co-localization with the mitochondrially anchored Venus-iLID-mito. A 30 s pulse of 37 W/m^2 blue (474nm) LED light source was used as a positive control.

(B) Data generated in Figure S1E was used to calculate the FZ dose dependent kinetics of CeNLuc activation. The signal half-lives for CeNLuc generated light output was calculated using a non-linear regression, one decay phase fit analysis in Prism.

(C) Based on half-life calculations from (B), 'pulsatile' activation of CeNLuc was performed using either a 1.25 or 0.625 μM FZ addition in order to generate light pulse every 15 or every 5 min (representative data, ± SEM, n = 3).

Figure S4. Live Cell Imaging Perfusion System, Related to Figure 4 and STAR Methods. A gravity driven FZ perfusion system was used to add furimazine/wash out furimazine in a pulsatile manner.

(A) Cells were seeded on a live cell imaging compatible slide that contained two ports (one for fluid intake and one for waste).

(B) The intake line was connected to two syringes (one for 20 μM FZ and one for Media only). See *Methods* section for additional details.

Figure S5. Amino Acid Sequences of LumiFluor Proteins Evaluated for Cellular Optogenetic Regulation.

GpNLuc (eGFP-NanoLuc fusion):

eGFP

Peptide Linker

NanoLuc

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIE
LKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPI
GDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKDISGGMVFTLED
FVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQ
MGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNG
NKIIDERLINPDGSLLFRVTINGVTGWRLCERILA*

CeNLuc (mCerulean3-NanoLuc fusion):

mCerulean3

Peptide Linker

NanoLuc

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVT
TLSWGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIE
LKGIDFKEDGNILGHKLEYNAIHGNVYITADKQKNGIKANFGLNCNIEDGSVQLADHYQQNTPI
GDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKDISGGMVFTLED
FVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQ

MGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNG
NKIIDERLINPDGSLLFRVTINGVTGWRLCERILA*

Nuclear-CeNLuc (NLS-mCerulean3-NanoLuc fusion):

2xNLS

mCerulean3

Peptide Linker

NanoLuc

MDPKKKRKVDPKKKRKVVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTL
KFICTTGKLPVPWPTLVTTLSWGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY
KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNAIHGNVYITADKQKNGIKANFGLNCNI
EDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGM
DELYKDISGGMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGEN
GLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEG
IAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILA*

OMMCeNLuc (T2-mCerulean3-NanoLuc fusion):

T2-Mitocondrial targeting tag

mCerulean3

Peptide Linder

NLuc

MVGRNSAIAAGVCGALFIGYCIYFDRKRRSDPNFKNRLRERRKKQKLAKERAGLSKLPDLKD
AEAVQKFGTVSKGEEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKL
PVPWPTLVTTLSWGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKF
EGDTLVNRIELKGIDFKEDGNILGHKLEYNAIHGNVYITADKQKNGIKANFGLNCNIEDGSVQL
ADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKDIS
GGMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVII
PYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKI
TVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILA*

Figure S6. Nucleotide Sequence and Plasmid Map of *8x gRNA* Promoter for Cellular Optogenetic Regulation of a *firefly* Luciferase Reporter.

8x gRNA – FLuc reporter:

8x gRNA binding sites

Minimal CMV promoter

***Firefly* luciferase**

CTGCA**AAAGGTCGAGAAACTGCAAAAGGTCGAGAAACTGCAAAAGGTCGAGAAACTGC**
AAAAGGTCGAGAAACTGCAAAAGGTCGAGAAACTGCAAAAGGTCGAGAAACTGCAAAAG
GTCGAGAAACTGCAAAAGGTCGAGAAACTGCAAAAGAGATCT**GGTAGGCGTGTACGGTGG**
GAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCAAGCTTGGCATTCCGGTA
CTGTTGGTAAACAAGTTTGTACAAAAAAGCAGGCTGCCACC**ATGGAAGACGCCAAAAACA**
TAAAGAAAGGCCCGGCGCCATTCTATCCGCTAGAGGATGGAACCGCTGGAGAGCAACT
GCATAAGGCTATGAAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACA
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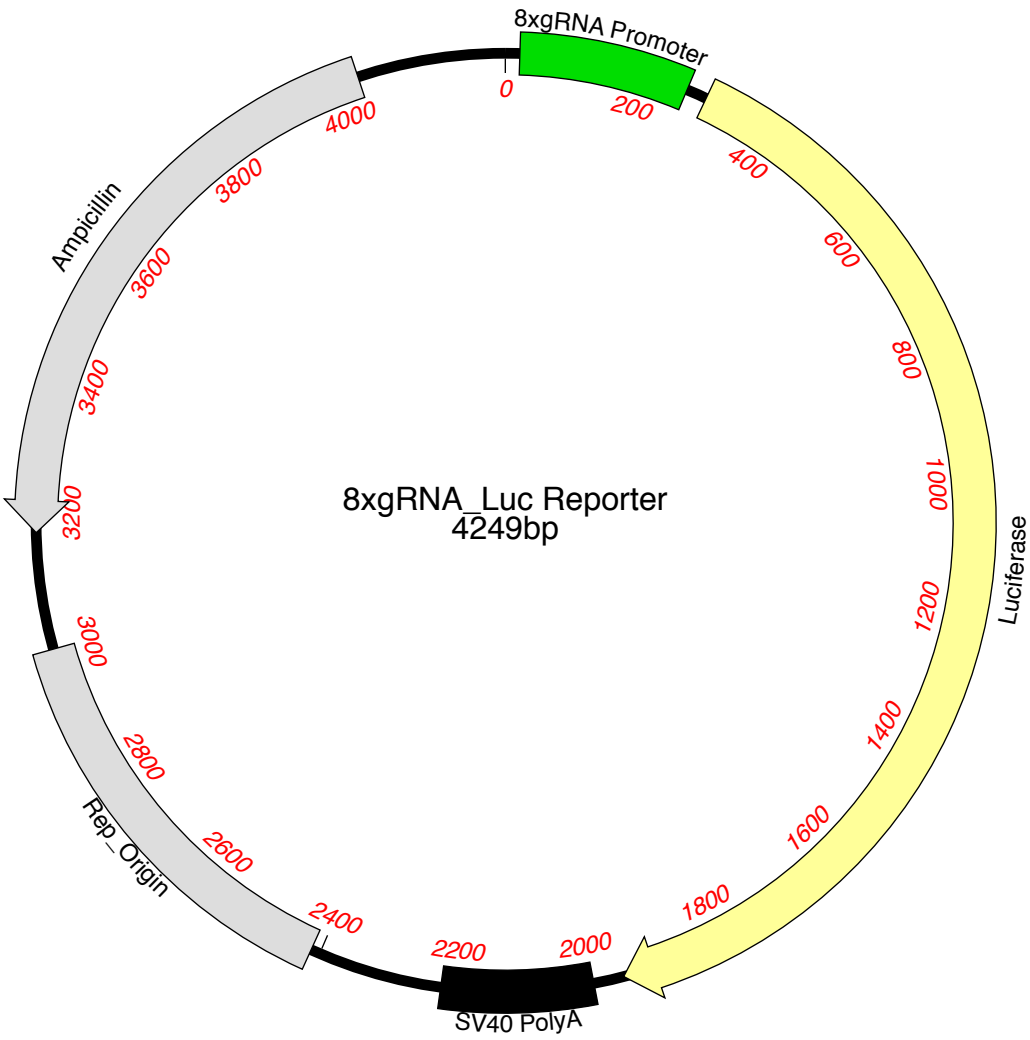

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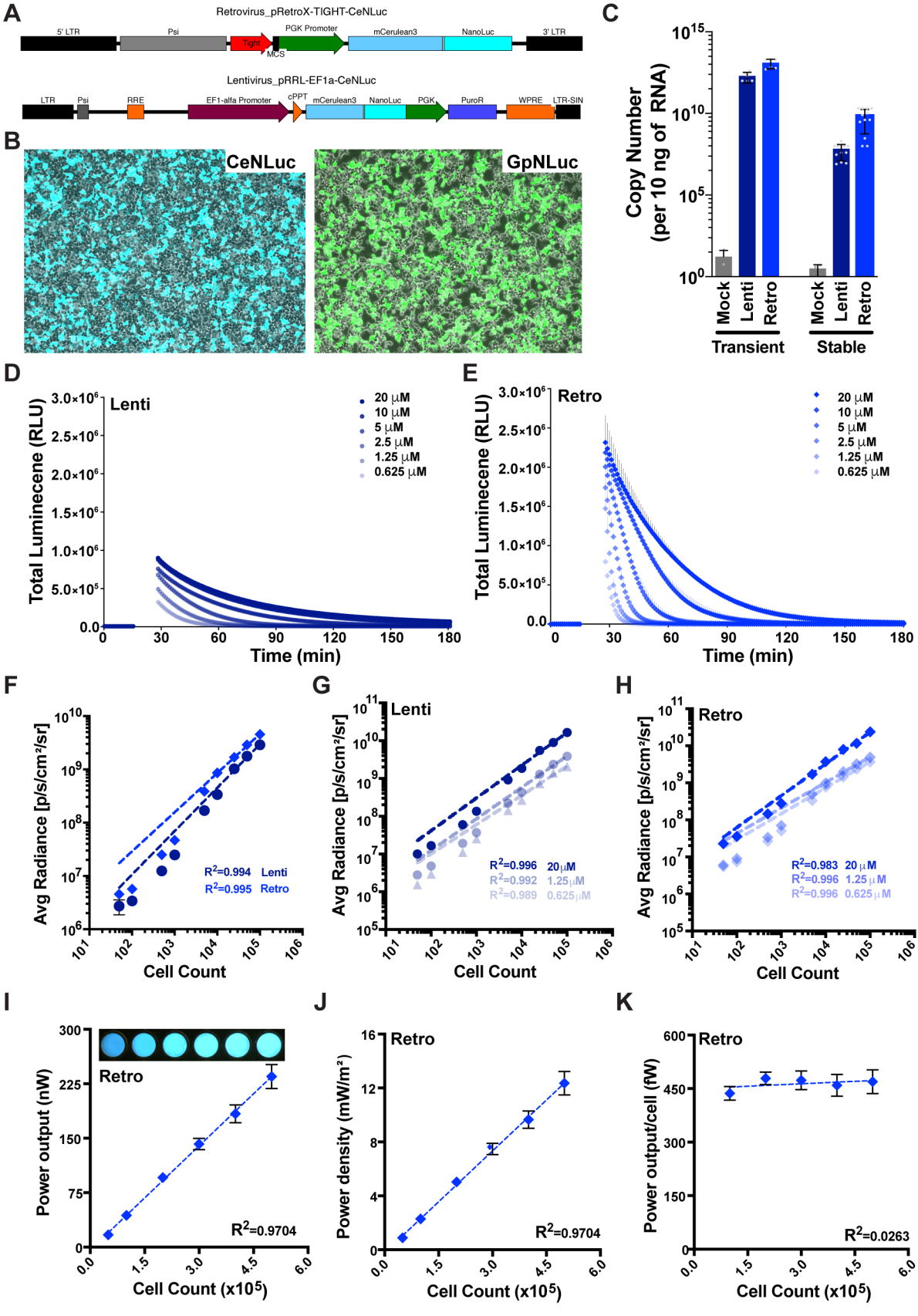
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5' LTR
Psi
Tight
PGK Promoter
MCS
mCerulean3
NanoLuc
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Lentivirus_pRRL-EF1a-CeNLuc
LTR
Psi
RRE
EF1-alfa Promoter
cPPT
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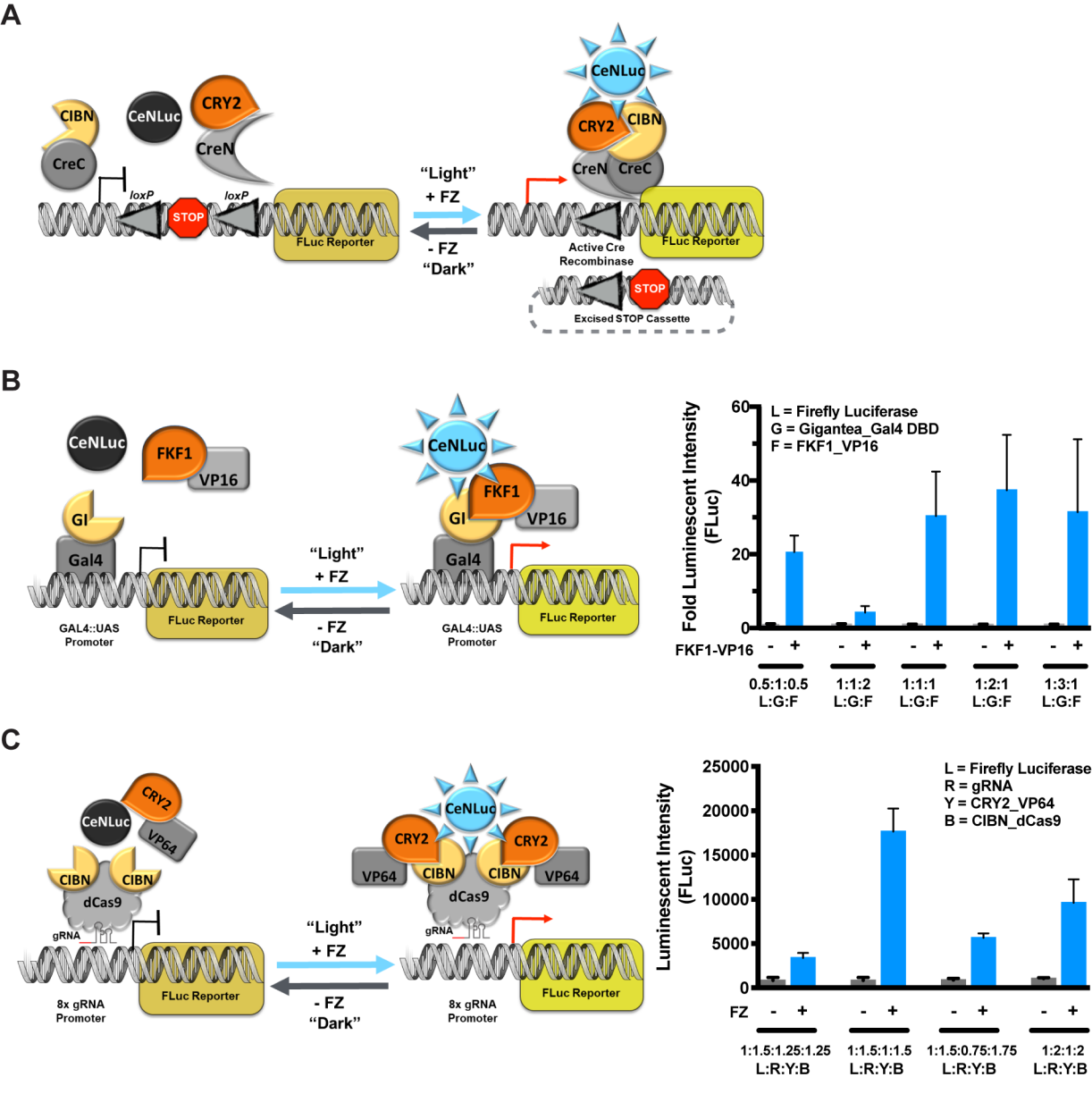
NanoLuc
PGK
PuroR
WPRE
LTR-SIN
B
CeNLuc
GpNLuc
C
Copy Number (per 10 ng of RNA)
Mock
Lenti
Retro
Transient
Stable
D
Lenti
Total Luminecene (RLU)
Time (min)
20 μM
10 μM
5 μM
2.5 μM
1.25 μM
0.625 μM
E
Retro
Total Luminecene (RLU)
Time (min)
F
Avg Radiance [p/s/cm²/sr]
Cell Count
R²=0.994 Lenti
R²=0.995 Retro
G
Lenti
R²=0.996 20μM
R²=0.992 1.25μM
R²=0.989 0.625μM
H
Retro
R²=0.983 20 μM
R²=0.996 1.25 μM
R²=0.996 0.625 μM
I
Retro
Power output (nW)
R²=0.9704
Cell Count (x10⁵)
J
Retro
Power density (mW/m²)
R²=0.9704
Cell Count (x10⁵)
K
Retro
Power output/cell (fW)
R²=0.0263
Cell Count (x10⁵)

Figure S1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CIBN
CeNLuc
CRY2
CreN
CreC
loxP
loxP
STOP
FLuc Reporter
"Light"
+ FZ
- FZ
"Dark"
CeNLuc
CRY2
CIBN
CreN
CreC
Active Cre
Recombinase
FLuc Reporter
STOP
Excised STOP Cassette
B
CeNLuc
FKF1
VP16
GI
Gal4
GAL4::UAS
Promoter
FLuc Reporter
"Light"
+ FZ
- FZ
"Dark"
CeNLuc
FKF1
GI
VP16
Gal4
GAL4::UAS
Promoter
FLuc Reporter
L = Firefly Luciferase
G = Gigantea_Gal4 DBD
F = FKF1_VP16
Fold Luminescent Intensity
(FLuc)
60
40
20
0
FKF1-VP16
- + - + - + - + - +
0.5:1:0.5
L:G:F
1:1:2
L:G:F
1:1:1
L:G:F
1:2:1
L:G:F
1:3:1
L:G:F
C
CeNLuc
CRY2
VP64
CIBN
CIBN
dCas9
gRNA
8x gRNA
Promoter
FLuc Reporter
"Light"
+ FZ
- FZ
"Dark"
CeNLuc
CRY2
CRY2
VP64
CIBN
CIBN
VP64
dCas9
gRNA
8x gRNA
Promoter
FLuc Reporter
L = Firefly Luciferase
R = gRNA
Y = CRY2_VP64
B = CIBN_dCas9
Luminescent Intensity
(FLuc)
25000
20000
15000
10000
5000
0
FZ
- + - + - + - +
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L:R:Y:B
1:1.5:1:1.5
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L:R:Y:B
1:2:1:2
L:R:Y:B

Figure S2

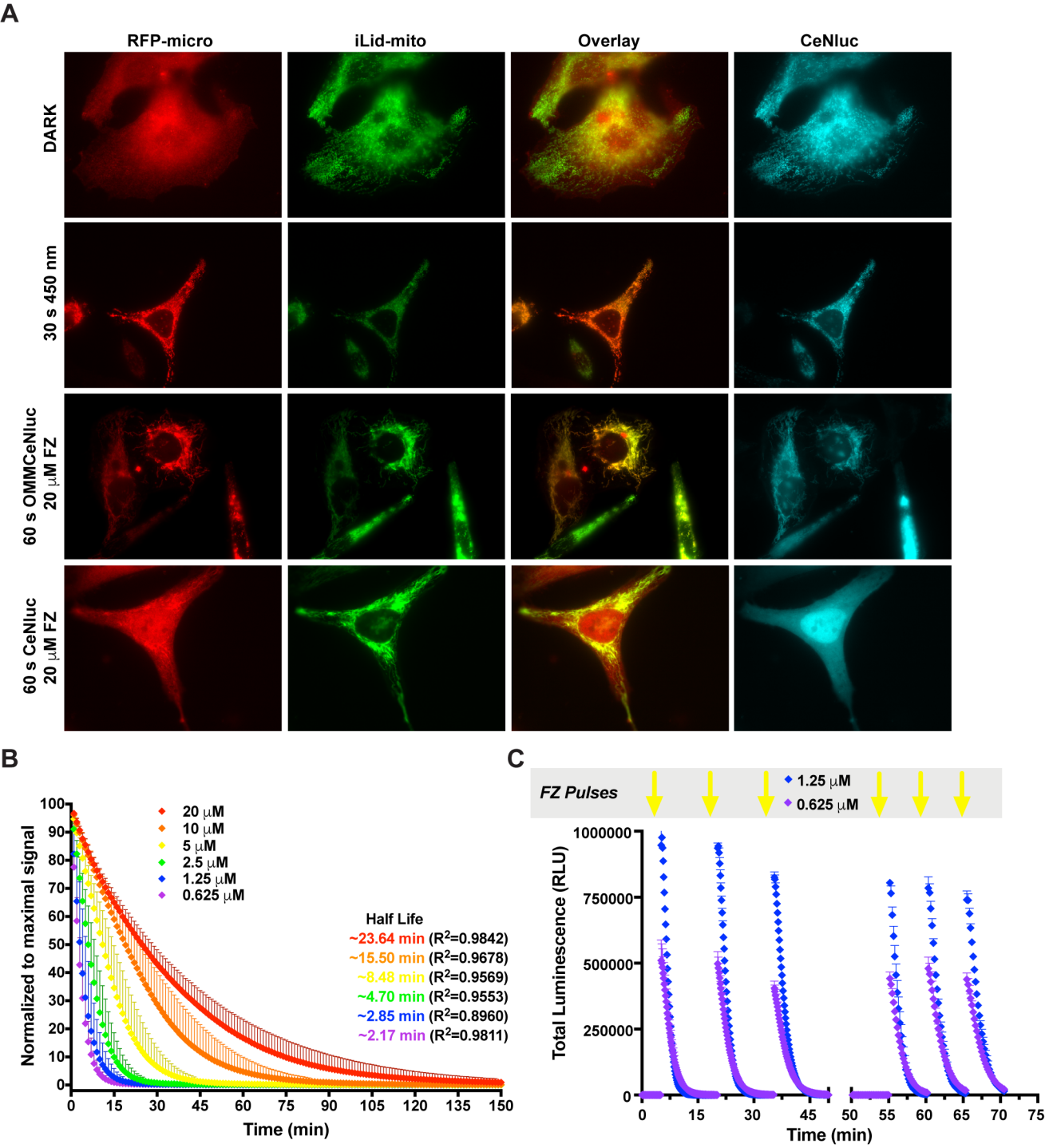
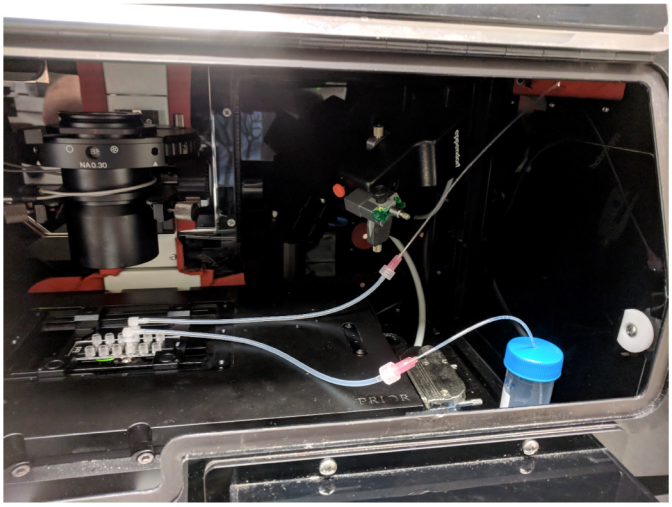

A
RFP-micro
iLid-mito
Overlay
CeNluc
DARK
30 s 450 nm
60 s OMMCeNluc 20 μM FZ
60 s CeNluc 20 μM FZ
B
Normalized to maximal signal
Time (min)
20 μM
10 μM
5 μM
2.5 μM
1.25 μM
0.625 μM
Half Life
~23.64 min (R2=0.9842)
~15.50 min (R2=0.9678)
~8.48 min (R2=0.9569)
~4.70 min (R2=0.9553)
~2.85 min (R2=0.8960)
~2.17 min (R2=0.9811)
C
FZ Pulses
1.25 μM
0.625 μM
Total Luminescence (RLU)
Time (min)

Figure S3

A



B

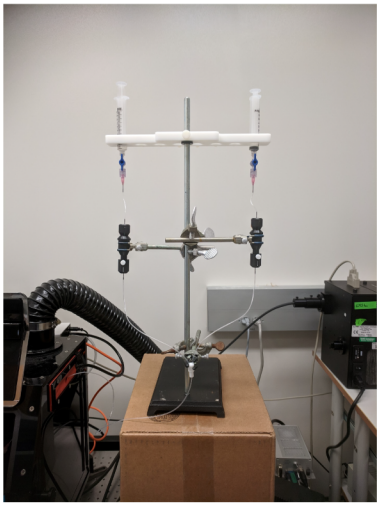


Figure S4