

Spatiotemporal control of RNA metabolism and CRISPR–Cas functions using engineered photoswitchable RNA-binding proteins

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SUPPLEMENTARY INFORMATION

Spatiotemporal control of RNA metabolism and CRISPR–Cas functions using engineered photoswitchable RNA-binding proteins

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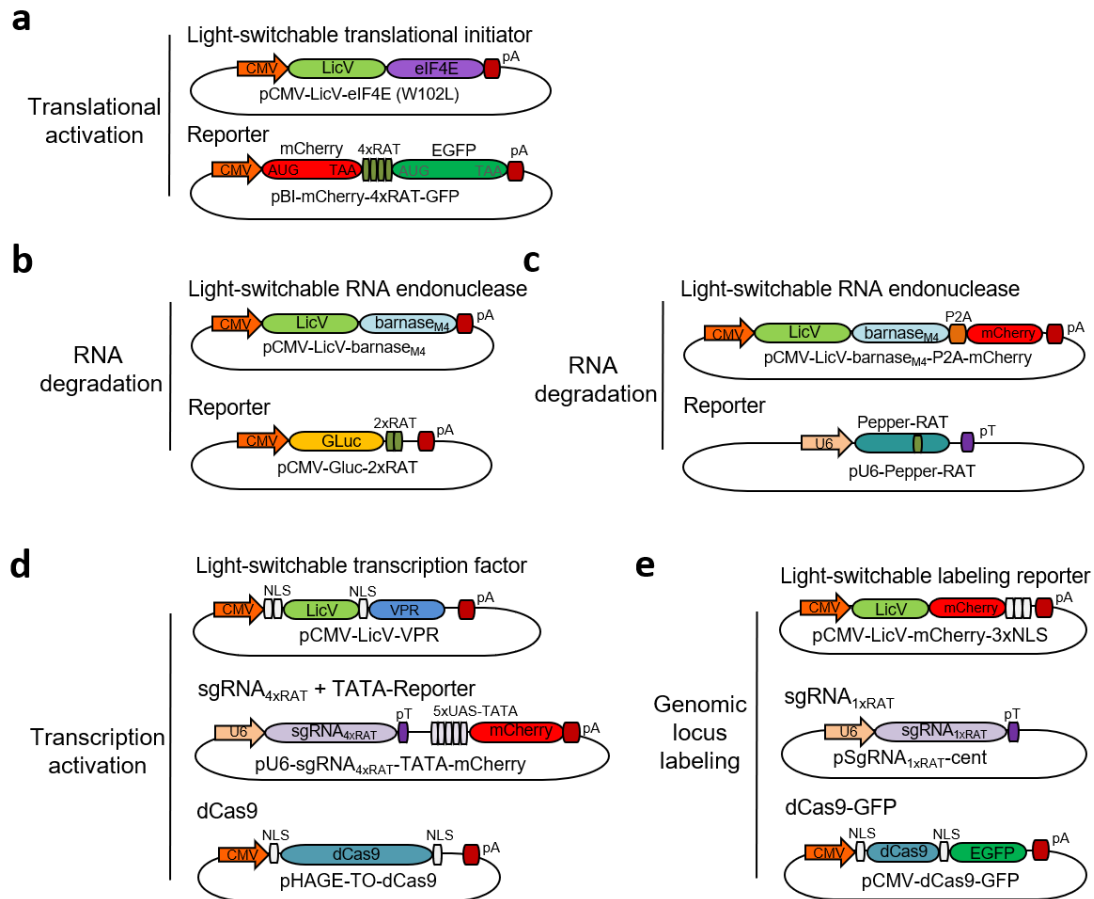
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Supplementary Note

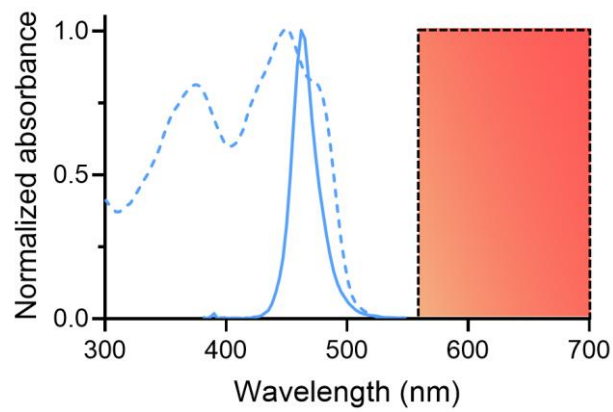
Sequence information of the constructs used in this protocol



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Supplementary Fig. 1 Schematic representation of the constructs expressing LicV-based light-switchable RNA effectors or the corresponding reporters. (a) Schematic representation of constructs for optogenetic control of mRNA translation, one of which encodes the light-switchable translational initiator LicV-eIF4E and the other encodes the bicistronic reporter containing four copies of RAT (4xRAT) upstream of EGFP mRNA. **(b)** Schematic representation of constructs for optogenetic control of RNA cleavage, one of which encodes the light-switchable endonuclease LicV-barnase_{M4} and the other encodes GLuc reporter mRNA containing two copies of RAT (2xRAT) located in the 3' UTR. **(c)** Schematic representation of constructs for optogenetic control of RNA cleavage using Pepper as the reporter, one of which encodes the light-switchable endonuclease LicV-barnase_{M4} and mCherry reporter separated by a P2A peptide cleavage site, and the other encodes Pepper reporter RNA containing one copy of RAT in its stem-loop. **(d)** Schematic representation of constructs for optogenetic control of transcription, which encode the light-switchable transcription factor LicV-VPR (LVPR), chimeric sgRNA containing four copies of RAT and mCherry reporter containing 5xUAS and TATA upstream of mCherry gene, and dCas9 protein, respectively. The ORF of mCherry reporter and the chimeric sgRNA expression cassette are integrated into one plasmid. **(e)** Schematic representation of constructs for optogenetic control of genomic locus labeling, which encode mCherry-tagged LicV, chimeric sgRNA containing one copy of RAT, and EGFP-tagged dCas9, respectively.

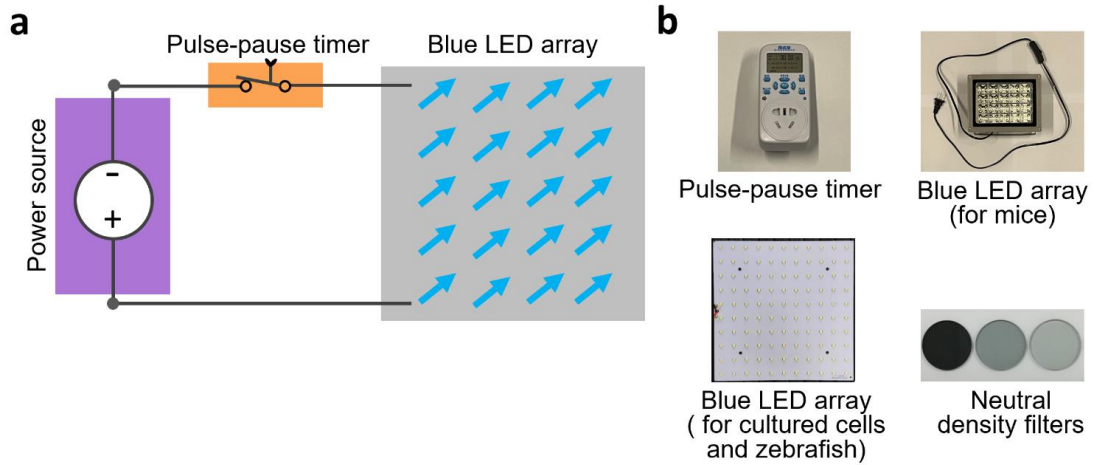
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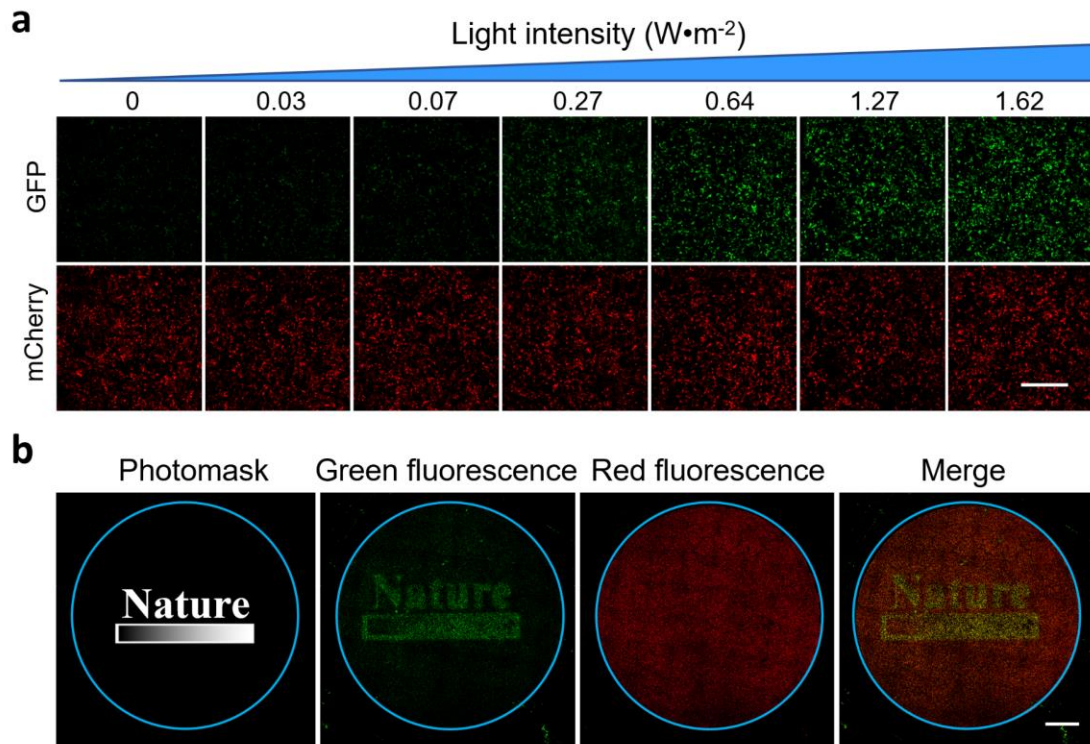
3 **Supplementary Fig. 2 Light-sensitivity of the LicV-based optogenetic systems.** Light-
4 sensitivity is determined by a combination of the absorption spectrum of the LicV in a ground
5 state (dashed blue line) and the emission of 460-nm blue LED arrays (solid blue line) used for
6 optogenetic activation in this protocol. The orange-red box highlights the spectral region that
7 is considered to be 'safe' in terms of a near-absence of LicV light-activation.

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Supplementary Fig. 3 Setup for the LED illumination. (a) The connection scheme of the major components is shown. To provide a pulsed regimen of illumination, the pulse-pause timer is serially connected between the LED array and the power source. **(b)** Photograph of the pulse-pause timer, blue LED arrays for cultured cells or mice, neutral density filters.



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2 **Supplementary Fig. 4 Quantitative and spatially resolved translation activation. (a)**

3 Quantitative activation of translation under blue light irradiance at different intensities. Scale

4 bar, 500 μm . **(b)** Spatially resolved translation activation. The transfected HEK293T cells were

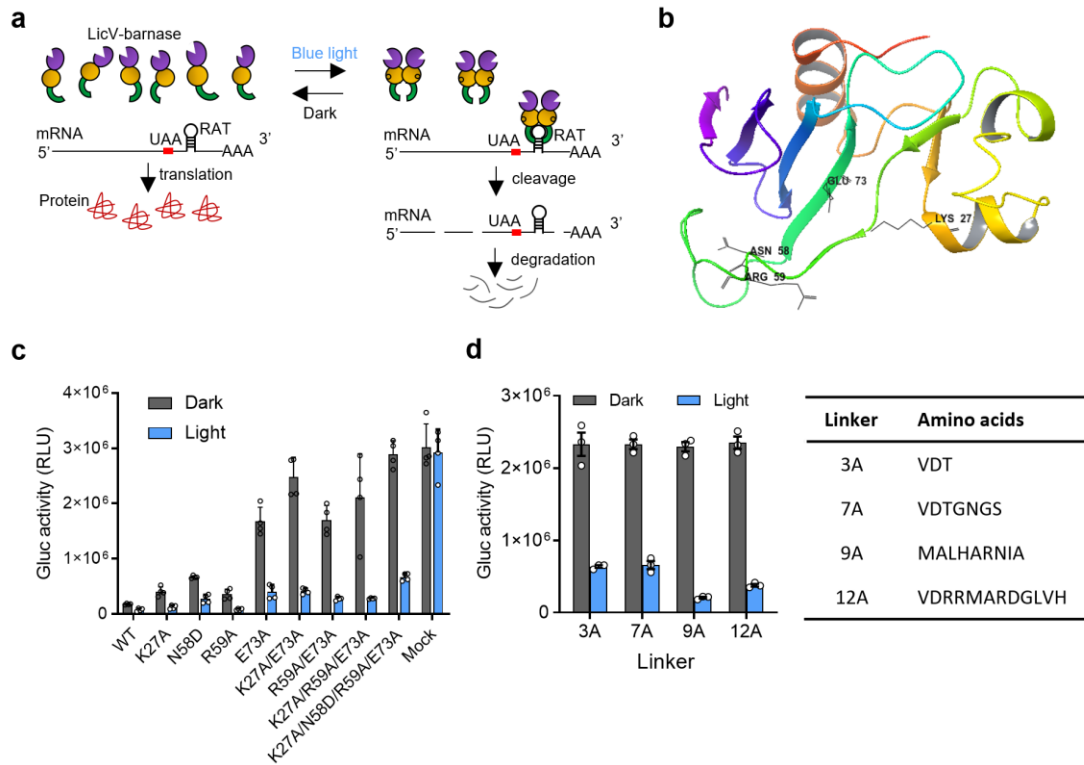
5 illuminated by blue light with a spatial pattern using a printed mask with a specific image (left

6 panel) for 24 h before the image of mCherry fluorescence was taken. The blue circle indicated

7 the glass bottom of the dish, where the cells were attached. Scale bar, 3 mm.

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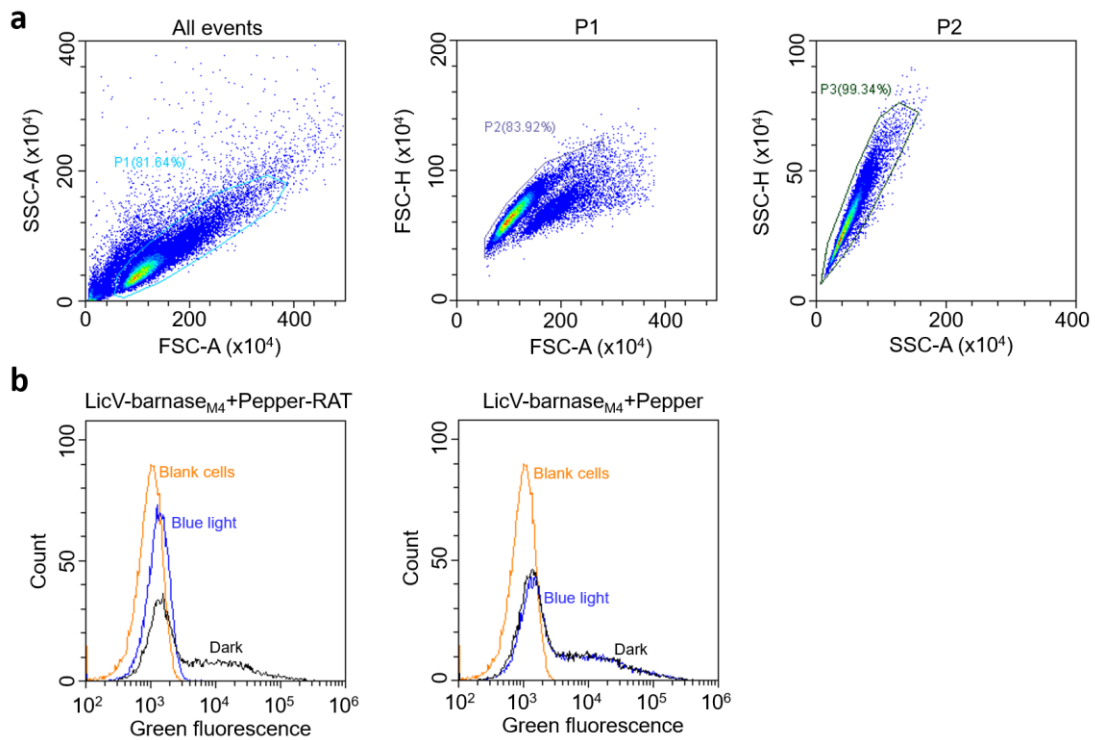


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2 **Supplementary Fig. 5 Optimization of the light-switchable RNA endonuclease. (a)** Schematic
 3 of light-induced specific cleavage of RNA by the light-switchable RNA endonuclease. **(b)** 3D
 4 structure information showing the mutation sites in barnase endonuclease to reduce its non-
 5 specific cleavage. PDB: 1BRS. **(c)** Measurement of the light-inducible cleavage of target RNA by
 6 different LicV-barnase variants. Data represent mean \pm s.d. from four biologically independent
 7 samples. **(d)** The effects of different linkers between LicV and barnase on the light-inducible
 8 cleavage of target RNA. Data represent mean \pm s.d. from three biologically independent
 9 samples. **a, c** adapted with permission from ref. 12. Source data for panels c and d is provided
 10 as Supplementary Data.

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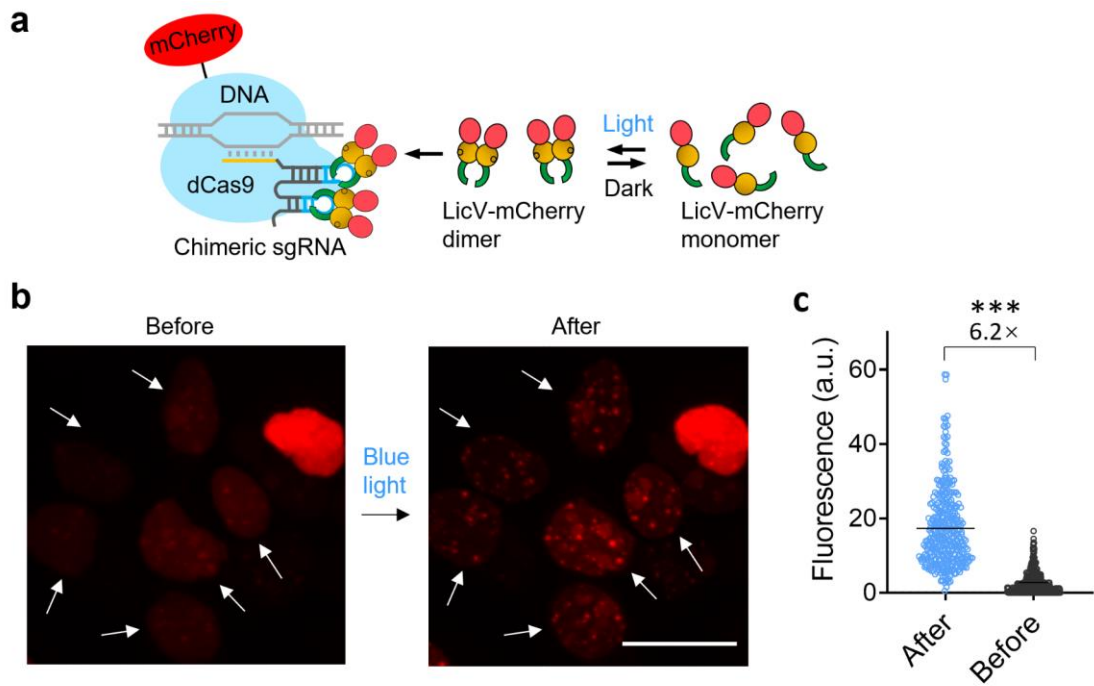
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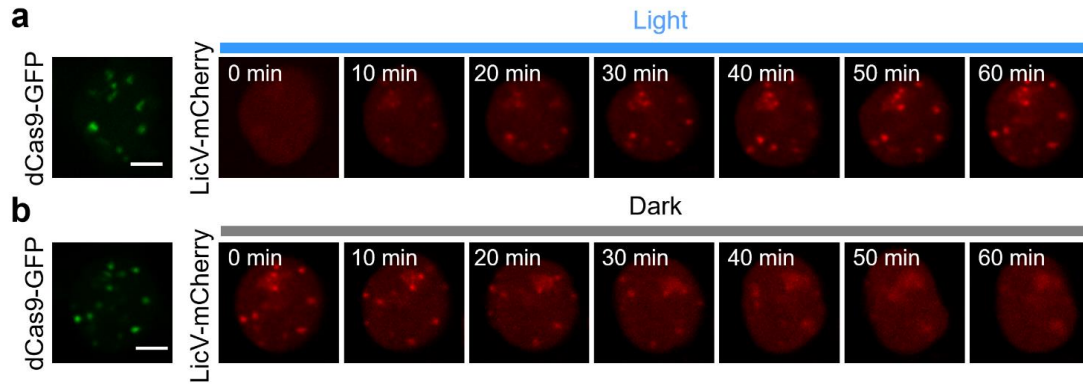
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Supplementary Fig. 6 FACS analysis of light-induced RNA degradation by LicV-barnase_{M4}. (a)

The gating strategy for the FACS analysis. (b) FACS analysis of Pepper fluorescence for the cells cultured under light or dark conditions. HEK293T cells coexpressing LicV-barnase_{M4} and Pepper-RAT were labeled with 0.2 μM HBC530 before analysis of Pepper530 fluorescence. Pepper alone without RAT sequence was used as the control. Blank cells incubated with the same HBC labeling solution were used to determine the population fraction that expresses the components of optogenetic systems.



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2 **Supplementary Fig. 7 Quantification of light-inducible recruitment of LicV-mCherry to the**
3 **centromere. (a)** Schematic representation of light-switchable recruitment of LicV-mCherry to
4 the chimeric sgRNA. **(b)** Fluorescence imaging of mCherry signals in the HEK293T cells
5 cotransfected with plasmids coexpressing LicV-mCherry, dCas9-mCherry and SgRNA_{4xRAT}
6 before and after blue light illumination. The enhanced fluorescence on the same centromere
7 after blue light illumination reflected the light-triggered recruitment of LicV-mCherry to the
8 centromeres. Scale bars, 20 μm. **(c)** Quantitative analysis of mCherry fluorescence intensity on
9 the centromeres of the cells. N= 362 puncta from 10 cells. Statistical comparison was
10 performed by a two-tailed *t* test. ***P < 0.001. The number of LicV-mCherry molecules
11 recruited to the centromere upon blue light induction was calculated using the following
12 equation: $N = \frac{F_{light} - F_{dark}}{F_{dark}}$, where F_{dark} and F_{light} represent mCherry fluorescence on
13 centromeres before and after blue light illumination. c adapted with permission from ref. 12.
14 Source data for panels c is provided as Supplementary Data.



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Supplementary figure 8 Kinetics of the light-inducible labeling of genomic foci. (a) Kinetics of the light-inducible recruitment of LicV_f-mCherry to the centromeres upon blue light illumination. Scale bar, 5 μ m. **(b)** Kinetics of the dissociation of LicV_f-mCherry from centromeres after removal of blue light. Scale bar, 5 μ m.

1 **Supplementary Note**

2 Sequence information of the constructs used in this protocol

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4 **pBI-mCherry-4xRAT-GFP**

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148

149 **pCMV-Gluc-2xRAT**

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220

221 **pCMV-LicV-barnase_{M4}**

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286

pU6-Pepper-RAT

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331

332 **pCMV-LicV-barnase_{M4}-P2A-mCherry**

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405

406 **pU6-sgRNA_{4xRAT}-TATA-mCherry**

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481

482 **pU6-sgRNA_{4xRAT}-TATA-NLuc**

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631

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676

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797

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