SUPPLEMENTARY MATERIAL

Engineering adenylate cyclase activated by near-infrared window light for mammalian optogenetic applications

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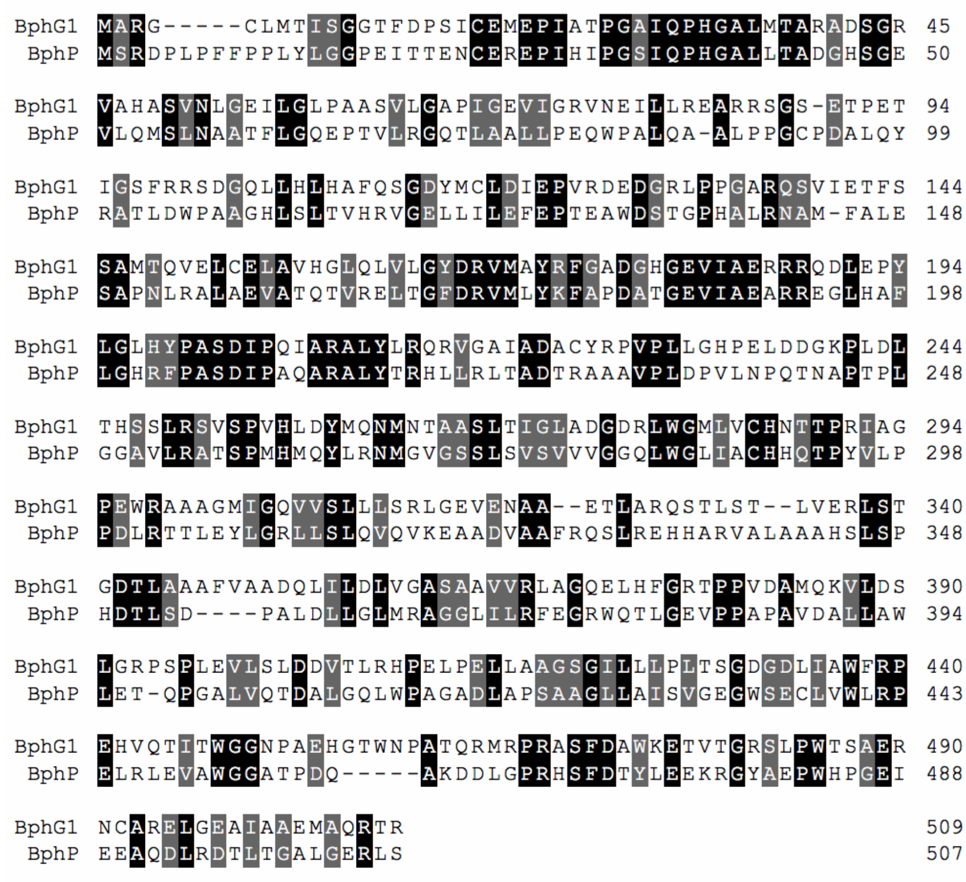
Supplementary Table 1. Bacterial strains and plasmids used in this study.

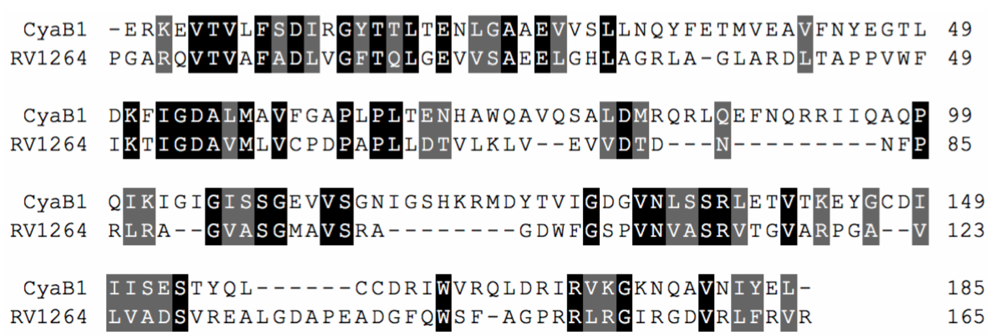
Strain or plasmid	Description	Reference or source
Escherichia coli		
DH5α	Strain for cloning and plasmid maintenance	NEB
BL21 [DE3]	Strain for protein overexpression	NEB
BL21 [DE3] *cya*	Strain for AC assays	[12]
Plasmids		
pET23a	Protein overexpression plasmid, P_{T7}, His_6-tag, Ap^R	Novagen
pET23::ilaC22_k27_Y259F	Plasmid for IlaC* overexpression, Ap^R	[12]
pET23::ilaD#	Plasmid for IlaD# overexpression, Ap^R	This study
pET23::ilaM#	Plasmid for IlaM# overexpression, Ap^R	This study
pT7-ho1	Plasmid for expression of heme oxygenase *ho1*	[10]
pAAV-hSyn-hChR2(H134R)-EYFP	AAV vector for expression of humanized ChR2 with H134R mutation fused to EYFP driven by P_{hSyn}	Addgene #26973
pAAV::ilaM5	AAV vector expressing IlaM5-p2A-BphO-p2A-eGFP under the hSyn promoter	This study
pAAV::ilaC*	AAV vector expressing IlaC*-p2A-BphO-p2A-eGFP under the hSyn promoter	This study
pCAG-GFP	Mammalian vector expressing eGFP under the CAG promoter	Addgene #11150
pCRE-MetLuc2	Mammalian cAMP reporter vector containing two copies of the cAMP-response element (CRE) fused to the TATA-like promoter controlling expression of the secreted *Metridia longa* luciferase gene	Clontech #631745

Supplementary Figure 1. Alignments of the (a) PSM and (b) AC modules used for NIRW-AC engineering. (a) Alignment of the PSM modules from *R. sphaeroides* BphG1 (aa 1-509) and *D. radiodurans* BphP (1-507). **(b)** Alignment of the catalytic AC domains from *Nostoc* sp. CyaB1 (aa 596-781) and *M. tuberculosis* RV1264 (aa 211-375). Identical residues are shown on the black background; similar residues on the grey background.

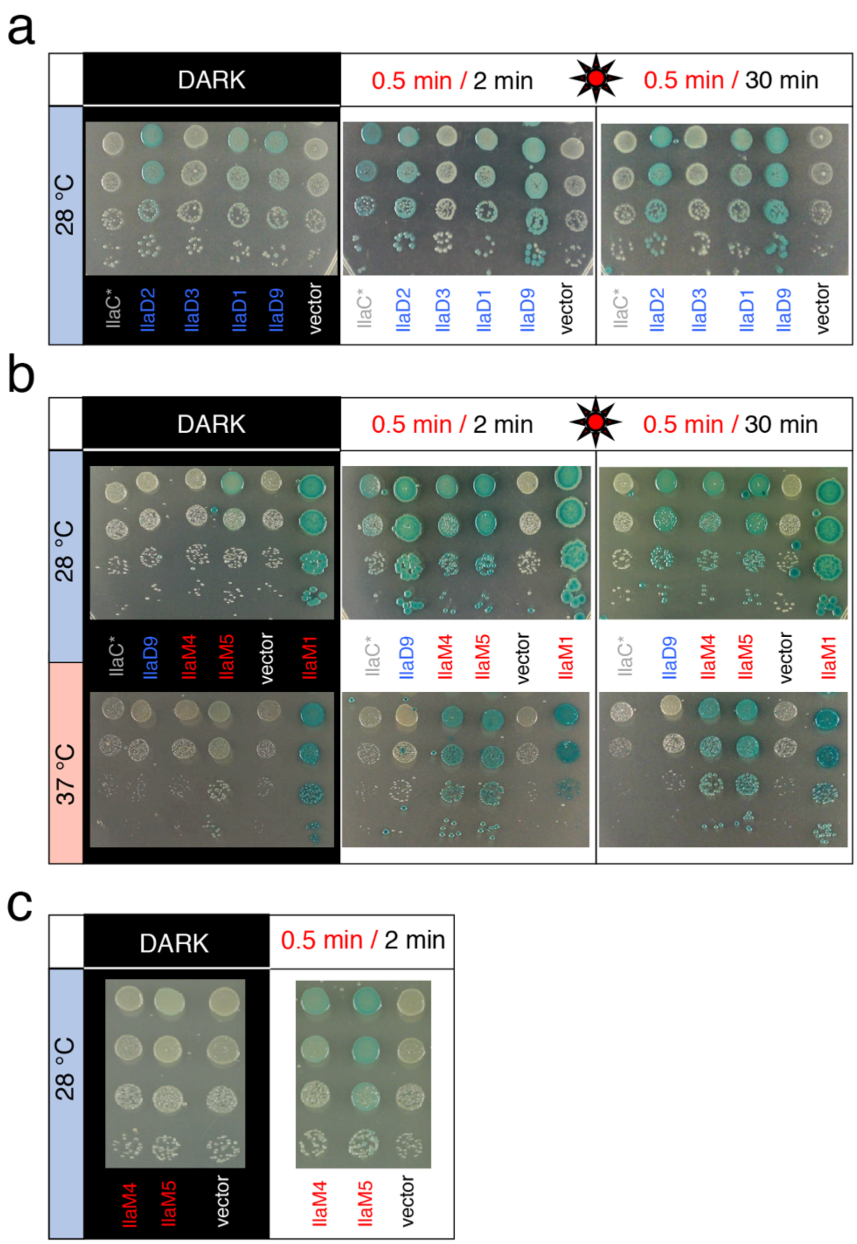
a



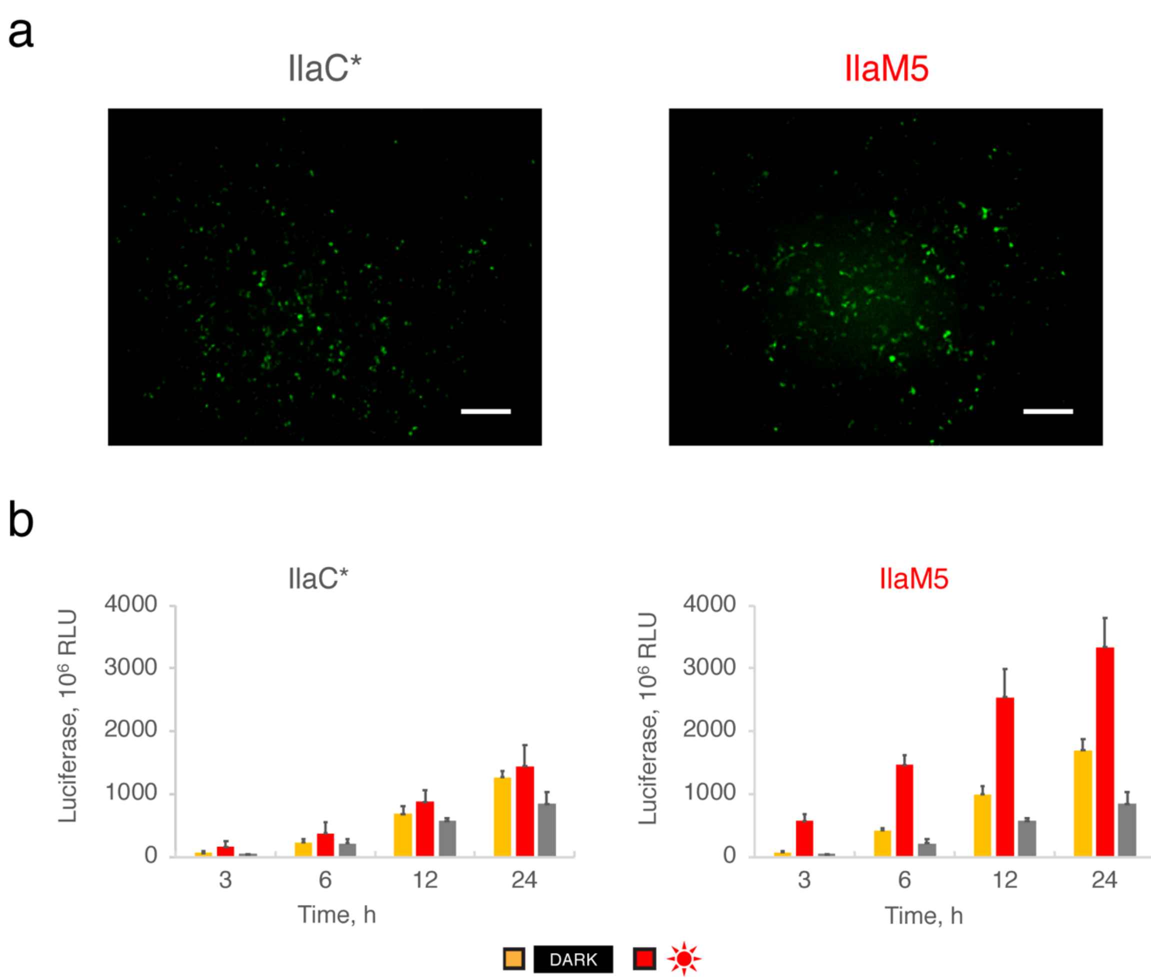
b



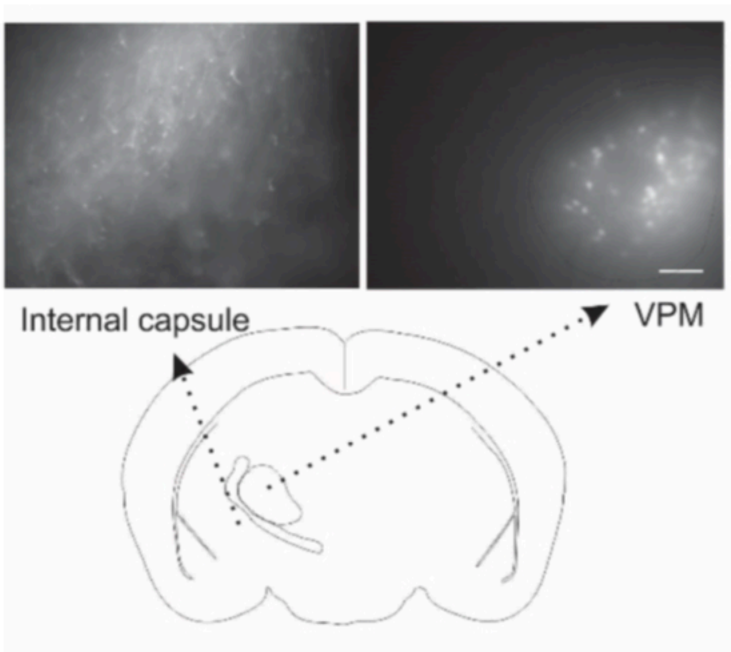
Supplementary Figure 2. Photoactivated cAMP-dependent gene expression in *E. coli* expressing engineered ACs. Shown are images of the β-galactosidase plate dilution assays indicative of cAMP levels. Dilutions of cultures of BL21[DE3] *cya* (pET::ila#; pT7-ho1) were spotted on LB agar containing Ap, Km, X-Gal and IPTG and grown at 28 ^{0}C or 37 ^{0}C either in the dark, or under red (660 nm) light. Two different irradiation regimens were used involving more frequent (0.5 min light/ 2 min dark) or less frequent (0.5 min light/ 30 min dark) light pulses. For growth in the dark, the plates were covered in aluminum foil. **(a)** Comparison of activities of IlaC* and ACs of the IlaD series at 28 ^{0}C (25 uM IPTG). **(b)** Comparison of activities of IlaC* and ACs of the IlaM series at 28 ^{0}C and 37 ^{0}C (25 uM IPTG). **(c)** Comparison of activities of IlaM4 and IlaM5 at 37 ^{0}C at lower expression levels (10 uM IPTG).



Supplementary Figure 3. Photoactivated cAMP-dependent gene expression in HEK293 cells expressing IlaC* or IlaM5. (a) Evidence of a functional hSyn promoter in the HEK293 cells. eGFP expressed from the hSyn promoter in the HEK293 cells transfected with pAAV::ilaC* and pCRE-MetLuc2 (left panel; Clonetech [TaKaRa, Catalog No. 631745) or pAAV::ilaM5 and pCRE-MetLuc2 (right panel), 48 h post-transfection. The pAAV::ilaC* and pAAV::ilaM5 constructs are shown in Fig. 5b. Fluorescent microscopy: excitation 488 nm, emission 540 nm. Scale bar, 160 μm. **(b)** Measurements of secreted MetLuc expressed from the cAMP-dependent promoter (pCRE-MetLuc2) in the HEK293 cells expressing IlaC* (pAAV::ilaC*) or IlaM5 (pAAV::ilaM5). Twenty-four h post-transfection the cells were exposed to pulsed (1 min light/ 5 min dark) red (660-nm) light (red bars) or grown in the dark for the duration of 3 to 24 h. Grey bars, MetLuc levels in the cells transfected with a negative control plasmid, pCAG-GFP, and pCRE-MetLuc2 and grown in the dark. These measurements represent accumulation of MetLuc due to native ACs. Shown are mean data ± SD from 3 independent experiments (2 technical replicates per experiment).



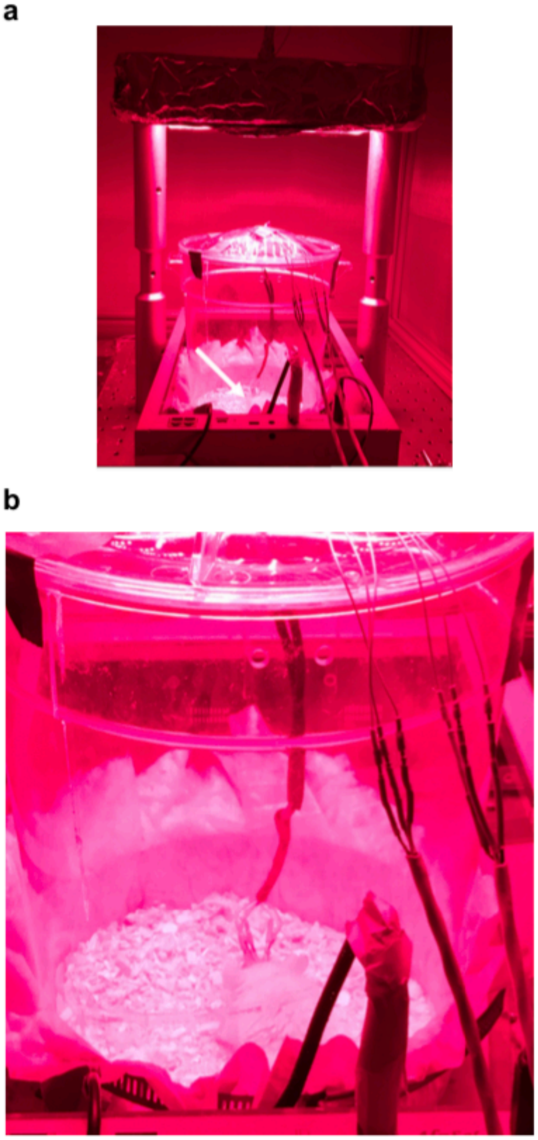
Supplementary Figure 4. Photostimulation of HCN currents by IlaM5 in thalamic neuron slices. Photomicrograph of thalamic slice in a pAAV::ilaM5 transfected mouse, showing robust eGFP expression from the hSyn promoter in thalamic relay neurons. Scale bar, 100 μm.



Supplementary Figure 5. Noninvasive NIRW light stimulation and EEG recording arena. (a) Photo of a mouse cage and a NIRW LED panel (300 W, nominal power) mounted approximately 30 cm above the cage floor. EEG recordings were taken via an electrode implanted into the mouse cortex or the thalamus region. White arrow points at a mouse. **(b)** Zoomed-in image of the cage shown in panel (a).

a

b



Supplementary Figure 6. Protein sequences of the engineered NIRW-ACs, IlaD9 and IlaM5. The sequence derived from BphP is shown in red; the sequence derived from CyaB1 is shown in light-blue; the sequence derived from Rv1264 is shown in navy-blue; the added residues to the α-helical linker are shown in green; the C-terminal His_6-tag is shown in black.

IlaD9

MSRDPLPFFPPLYLGGPEITTENCEREPIHIPGSIQPHGALLTADGHSGEVLQMSLNAATFLGQE
PTVLRGQTLAALLPEQWPALQAALPPGCPDALQYRATLDWPAAGHLSLTVHRVGELLILEFEPT
EAWDSTGPHALRNAMFALESAPNLRALAEVATQTVRELTGFDRVMLYKFAPDATGEVIAEARR
EGLHAFLGHRFPASDIPAQARALYTRHLLRLTADTRAAAVPLDPVLNPQTNAPTPLGGAVLRAT
SPMHMQYLRNMGVGSSLSVSVVVGGQLWGLIACHHQTPYVLPPDLRTTLEYLGRLLSLQVQV
KEAADVAAFRQSLREHHARVALAAAHSLSPHDTLSDPALDLLGLMRAGGLILRFEGRWQTLGE
VPPAPAVDALLAWLETQPGALVQTDALGQLWPAGADLAPSAAGLLAISVGEGWSECLVWLRP
ELRLEVAWGGATPDQAKDDLGPRHSFDTYLEEKRGYAEPWHPGEIEEAQDLRDTLTGALGER
LRAELERKEVTVLFSDIRGYTTLTENLGAAEVVSLLNQYFETMVEAVFNYEGTLDKFIGDALMAV
FGAPLPLTENHAWQAVRSALDMRQRLKEFNQRRIIQAQPQIKIGIGISSGEVVSGNIGSHKRMD
YTVIGDGVNLSSRLETVTKEYGCDIILSEFTYQLCSDRIRVRQLDKIRVKGKHQAVNIYELISDRS
TPLDDNTQEFLFHYHNGRTAYLVRDFTQAIACFNSAKHIRPTDQAVNIHLERAYNYQQTPPPPQ
WDGVWTIFTKHHHHHH

IlaM5

MSRDPLPFFPPLYLGGPEITTENCEREPIHIPGSIQPHGALLTADGHSGEVLQMSLNAATFLGQE
PTVLRGQTLAALLPEQWPALQAALPPGCPDALQYRATLDWPAAGHLSLTVHRVGELLILEFEPT
EAWDSTGPHALRNAMFALESAPNLRALAEVATQTVRELTGFDRVMLYKFAPDATGEVIAEARR
EGLHAFLGHRFPASDIPAQARALYTRHLLRLTADTRAAAVPLDPVLNPQTNAPTPLGGAVLRAT
SPMHMQYLRNMGVGSSLSVSVVVGGQLWGLIACHHQTPYVLPPDLRTTLEYLGRLLSLQVQV
KEAADVAAFRQSLREHHARVALAAAHSLSPHDTLSDPALDLLGLMRAGGLILRFEGRWQTLGE
VPPAPAVDALLAWLETQPGALVQTDALGQLWPAGADLAPSAAGLLAISVGEGWSECLVWLRP
ELRLEVAWGGATPDQAKDDLGPRHSFDTYLEEKRGYAEPWHPGEIEEAQDLRDTLTGALGER
LRAELAERKEVTVAFADLVGFTQLGEVVSAEELGHLAGRLAGLARDLTAPPVWFIKTIGDAVML
VCPDPAPLLDTVLKLVEVVDTDNNFPRLRAGVASGMAVSRAGDWFGSPVNVASRVTGVARPG
AVLVADSVREALGDAPEADGFQWSFAGPRRLRGIRGDVRLFRVRRGATRTGSGGAAQDDDL
AGSSPHHHHHH