Extended Data

Labeling and imaging of neurons with a genetically encoded autonomous bioluminescence system

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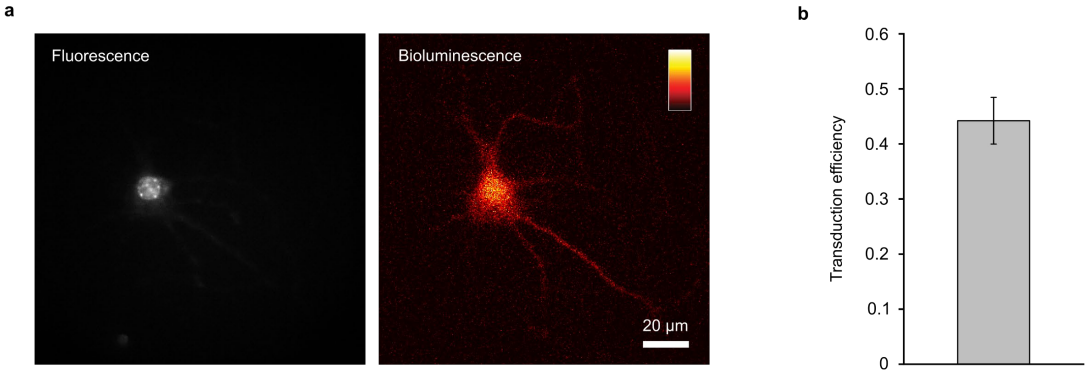
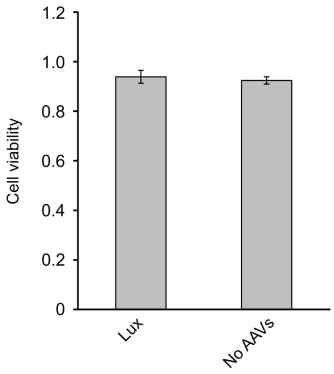
Extended Data Table 1. Primers used for plasmid cloning.

Insert	Primer Name	Primer Sequence (5'→3')
CBh promoter	CBh MluI fwd	TTGATCACGCGTCGTTACATAACTTACGGT
	CBh KpnI rev	TTACTAGGTACCCCAACCTGAAAAAAAGTGATTTCAGG
CAG promoter	CAG MluI fwd	TTGATCACGCGTGCGTTACATAACTTACGGTAAATGG
	CAG KpnI rev	ATGTAAGGTACCCGCCCGCCG
CMV promoter	CMV MluI fwd	TGAATAACGCGTGTAATCAATTACGGGGTC
	CMV KpnI rev	ATGTAAGGTACCTGCTTATATAGACCTCCC
Cofilin-Citrine	Cofilin NheI fwd	AAGATTGGATCCGCTAGCATGGCCTCTGGTGTGGCTGTC
	Cofilin AgeI rev	TAACTGACCGGTGCGTCCCCCAAAGGCTTGCCCTCCAG
	Citrine AgeI fwd	TCCACCGGTCGCCACCATGGTGAGCAAGGGCGAG
	Citrine AscI rev	TGAATGGAATTCGGCGCGCCTTACTTGTACAGCTCGTCC
EGFP	EGFP NheI fwd	CGACGCTAGCATGGTGAGCAAGGGCG
	EGFP AscI rev	TGAATGGAATTCGGCGCGCCTTACTTGTACAGCTCGTCC
Htt(25Q)-Citrine	Htt NheI fwd	GTTGCAGCTAGCATGGCGACCCTGGAA
	Citrine AscI rev	CTTATAGGCGCGCCTTACTTGTACAGCTCGTCC
Htt(73Q)-Citrine	Htt NheI fwd	GTTGCAGCTAGCATGGCGACCCTGGAA
	Citrine AscI rev	CTTATAGGCGCGCCTTACTTGTACAGCTCGTCC
LuxA	LuxA NheI fwd	GTTAGTGCTAGCATGAAGTTCGGCAACTTCCTG
	LuxA AscI rev	CTTATAGGCGCGCCTCAGTACAGCAGGCTGCG
LuxB	LuxB NheI fwd	TAAGTCGCTAGCATGAAGTTCGGCCTGTTC
	LuxB AscI rev	CTTATAGGCGCGCCTCAGGTGTACTCCATGTGGT
LuxC	LuxC NheI fwd	CGAAGTGCTAGCATGACCAAGAAGATCAGCTTC
	LuxC AscI rev	CTTATAGGCGCGCCTCAGGGCACGAACACCAG
LuxD	LuxD NheI fwd	TGAATAGCTAGCATGGAGAACGAGAGCAAGTACAAGAC
	LuxD AscI rev	CTTATAGGCGCGCCTCAGCTCAGGCTGATGGC
LuxE	LuxE NheI fwd	GTTAGTGCTAGCATGACCAGCTACGTGGACA
	LuxE AscI rev	CTTATAGGCGCGCCTCAGCTGTCGAAGGCCTC
Frp	Frp NheI fwd	CGTTAGGCTAGCATGGTGAAGATCCAGCCCATCCCCACCACC
	Frp AscI rev	CTTATAGGCGCGCCTCAGCGCTTGGCCAGGC
LuxB-P2A-luxA	LuxB NheI fwd	TAAGTCGCTAGCATGAAGTTCGGCCTGTTC
	LuxA AscI rev	CTTATAGGCGCGCCTCAGTACAGCAGGCTGCG
LuxD-P2A-luxC	LuxC P2A BamHI fwd	GTGAATGGATCCGGCGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCCATGACCAAGAAGATCAGCTTCATC
	LuxC AscI rev	CTTATAGGCGCGCCTCAGGGCACGAACACCAG

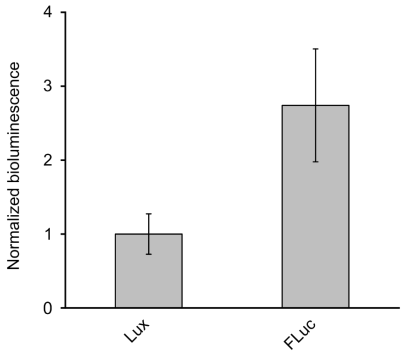
LuxD-P2A-luxE	LuxD NheI fwd	TGAATAGCTAGCATGGAGAACGAGAGCAAGTACAAGAC
	LuxE AscI rev	CTTATAGGCGCGCCTCAGCTGTCGAAGGCCTC
LuxE-T2A-frp	LuxE NheI fwd	GTTAGTGCTAGCATGACCAGCTACGTGGACA
	T2A ApaI rev	TCATTGGGGCCCGGGGTTCTCCTCCACGTCGCCGCA
	Frp ApaI fwd	TGAATAGGGCCCATGGTGAAGATCCAGCCC
	Frp AscI rev	CTTATAGGCGCGCCTCAGCGCTTGGCCAGGC



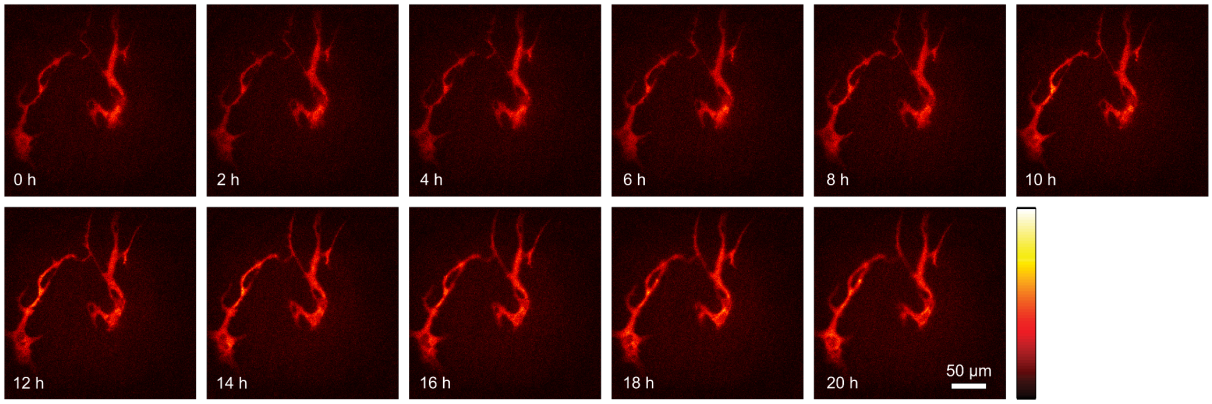
Extended Data Figure 1. Neuronal transduction efficiency of Lux AAVs. **(a)** Neurons were transduced with Lux AAVs. 6 days post-transduction, cells were labeled with Hoechst 33342 and fluorescence images with excitation at 405 nm were recorded (left). Bioluminescence images of the same cells were acquired with an exposure time of 1 min (right). The colormaps were scaled to the minimum and maximum pixel values of the images. **(b)** The transduction efficiency was determined as the fraction of bioluminescent cells. The error bar represents the standard deviation of 5 coverslips of cells.



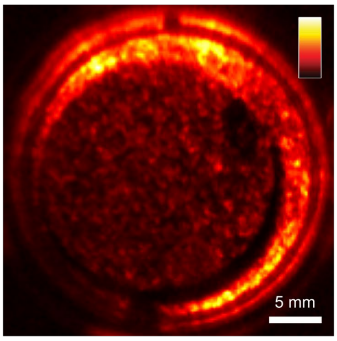
Extended Data Figure 2. Effect of Lux expression on neuronal viability. Neurons were transduced with Lux AAVs. 6 days post-transduction, cells were labeled with both propidium iodide and Hoechst 33342 to determine the number of dead cells and the total cell number, respectively. Fluorescence images were recorded with an IX83 microscope. Cell viability was calculated as the fraction of propidium iodide-negative cells. Neurons without AAV treatment were used for comparison. Error bars represent standard deviation of 6 coverslips of cells.



Extended Data Figure 3. Comparison of Lux and FLuc. Neurons were transduced with Lux AAVs or AAVs containing FLuc and bioluminescence was recorded 7 days post-transduction using a CLARIOstar Plus microplate reader. For FLuc, D-luciferin was added to the medium at a concentration of 300 µg/ml. Error bars represent standard deviation of 6 wells of cells.



Extended Data Figure 4. Temporal stability of the bioluminescence signal of neurons transduced with Lux AAVs. Imaging was started 6 days post-transduction. The colormap was scaled to the minimum and maximum pixel value of the image series.



Extended Data Figure 5. Bioluminescence image of neurons in a well of a 12-well plate. Neurons were transduced with Lux AAVs and imaged with an AI680RGB 8 days post-transduction. The image was recorded at the lower tray position with an exposure time of 5 min.