

Supporting Information

Increased autonomous bioluminescence emission from mammalian cells by enhanced cofactor synthesis

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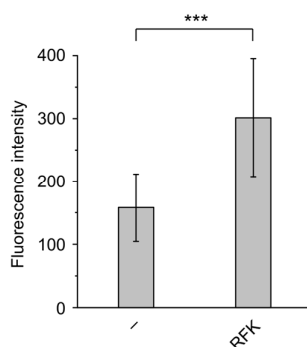


Figure S1. Fluorescence of phiLOV2.1-expressing HeLa cells with and without RFK. Cells on coverslips were transfected with a mixture of 0.8 μ g phiLOV2.1 pcDNA3.1(+) and 0.2 μ g RFK pcDNA3.1(+) or the empty pcDNA3.1(+) vector (-). Fluorescence was recorded with an IX83 widefield microscope. Error bars represent standard deviation from at least 20 cells. *** represents a p value of <0.001 , calculated by a 2-tailed Student's t test.

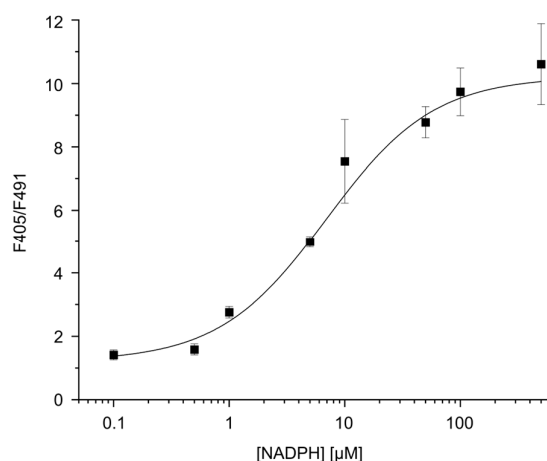


Figure S2. Calibration curve of iNap1. Fluorescence of purified iNap1 protein upon excitation at 405 (F405) and 491 nm (F491) was recorded at different NADPH concentrations. Data points and error bars represent average values and standard deviations of 5 measurements. The measured values were fitted with a 4-parameter logistic (4PL) curve (shown as a line).

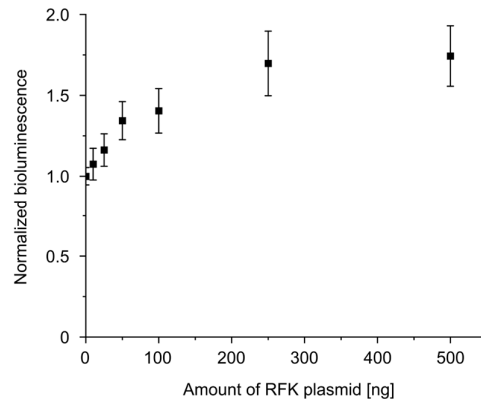


Figure S3. Bioluminescence emission of LiveLight HEK293 cells with different expression levels of RFK. Cells were grown in 24-well plates and transfected with the indicated amounts of RFK pcDNA3.1(+). Empty pcDNA3.1(+) vector was added up to a total DNA amount of 500 ng per well. The signal was normalized to cells transfected with 500 ng of the empty pcDNA3.1(+) vector. Error bars represent standard deviation from 5 wells.

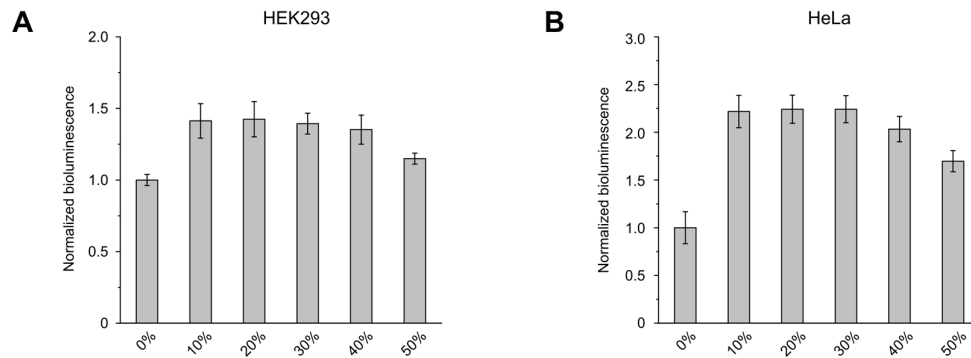


Figure S4. Brightness of cells transfected with lux plasmids and Akt2CA-P2A-RFK in different ratios. (A) HEK293 and (B) HeLa cells were grown in 24-well plates and transfected with a total amount of 0.5 μ g DNA with the indicated percentage of the Akt2CA-P2A-RFK plasmid. Error bars represent standard deviation of 5 wells.

Table S1. Primers used for plasmid construction.

Gene	Primer Name	Sequence (5'→3')
RFK	RFK NheI fwd	TGTAAAGCTAGCATGAGGCACCTGCCTTACTTC
	RFK XhoI rev	AATGTACTCGAGTCAGTGGCCATTCAATTATTTT
G6PD	G6PD NheI fwd	TGTAATGCTAGCATGGGTGCATCGGGTGAC
	G6PD XhoI rev	TGTAATCTCGAGTCAGAGCTTGTGGGGGTTTCAC
SIRT2	SIRT2 NheI fwd	AGTTTAGCTAGCATGGACTTCCTGCGGAAC
	SIRT2 XhoI rev	ATTGTACTCGAGTCACTGGGGTTTCTCCCT
NADK	NADK NheI fwd	ATGTAAGCTAGCATGGAAATGGAACAAGAAAAA ATGA
	NADK BamH rev	GTAATAGGATCCCTAGCCCTCCTCCTCCTC
Akt1	Akt1 NheI fwd	AGTAATGCTAGCATGAGCGACGTGGCTATT
	Akt1 XhoI rev	TGTAATCTCGAGTCAGGCCGTGCCGCTGGC
Akt2	Akt2 NheI fwd	TGAATAGCTAGCATGAATGAGGTGTCTGTCAT
	Akt2 XhoI rev	TGAATACTCGAGTCACTCGCGGATGCTGGC
Akt1CA	myr-Akt1 NheI fwd	TGAATAGCTAGCATGGGTTCTTCCAAATCCAAGC CCAAGGCAAGCGCCATGATGAGCGACGTGGCTAT TGTG
	Akt1 XhoI rev	TGTAATCTCGAGTCAGGCCGTGCCGCTGGC
Akt2CA	myr-Akt2 NheI fwd	TGAATAGCTAGCATGGGTTCTTCCAAATCCAAGC CCAAGGCAAGCGCCATGATGAATGAGGTGTCTGT CATCAAAGA
	Akt2 XhoI rev	TGAATACTCGAGTCACTCGCGGATGCTGGC
Akt2CA-P2A-RFK	myr-Akt2 NheI fwd	TGAATAGCTAGCATGGGTTCTTCCAAATCCAAGC CCAAGGCAAGCGCCATGATGAATGAGGTGTCTGT CATCAAAGA
	Akt2 BamHI rev	AATGTAGGATCCCTCGCGGATGCTGGCCGA
	P2A-RFK BamHI fwd	TTTTAGTGGATCCGGCGCCACCAACTTCAGCCTGC TGAAGCAGGCCGCGACGTGGAGGAGAACCCCG GCCCCATGAGGCACCTGCCTTACTTC
	RFK XhoI rev	AATGTACTCGAGTCAGTGGCCATTCAATTATTTT
RFK-P2A-Akt2CA	RFK NheI fwd	TGTAAAGCTAGCATGAGGCACCTGCCTTACTTC
	RFK BamHI rev	AATGTAGGATCCGTGGCCATTCAATTATTTTGCTTTT A
	P2A-myr-Akt2 BamHI fwd	TGAGTTGGATCCGGCGCCACCAACTTCAGCCTGC TGAAGCAGGCCGCGACGTGGAGGAGAACCCCG GCCCCATGGGTTCTTCCAAATCCAAGCCC
	Akt2 XhoI rev	TGAATACTCGAGTCACTCGCGGATGCTGGC
phiLOV2.1	phiLOV BamHI fwd	TTATCAGGATCCATGGGTCCACTGGGCAGC
	phiLOV XhoI rev	TCTAAGCTCGAGTTAGACGTGATCGCTACC
His-iNap1	His-iNap1 BamHI fwd	GTAATGGGATCCATGAGAGGATCGCATCACCATC ACCATCACGGTTCTATGAACCGGAAGTGGGGC
	iNap1 Sall rev	AGTTACGTCGACCTAGCCCATCATCTCCTCCC

Table S2. NADPH concentrations in Lux-expressing cells with and without Akt2CA expression. Cells were transfected and imaged as indicated in Figure 3. Cellular NADPH concentrations were calculated from the fluorescence ratio F405/F491 using the calibration curve shown in Figure S1.

Cell line	Transfection	[NADPH] [μ M]
LiveLight HEK293	-Akt2CA	9.3
	+Akt2CA	35.8
HEK293	-Akt2CA	9.7
	+Akt2CA	14.0
HeLa	-Akt2CA	4.4
	+Akt2CA	11.8