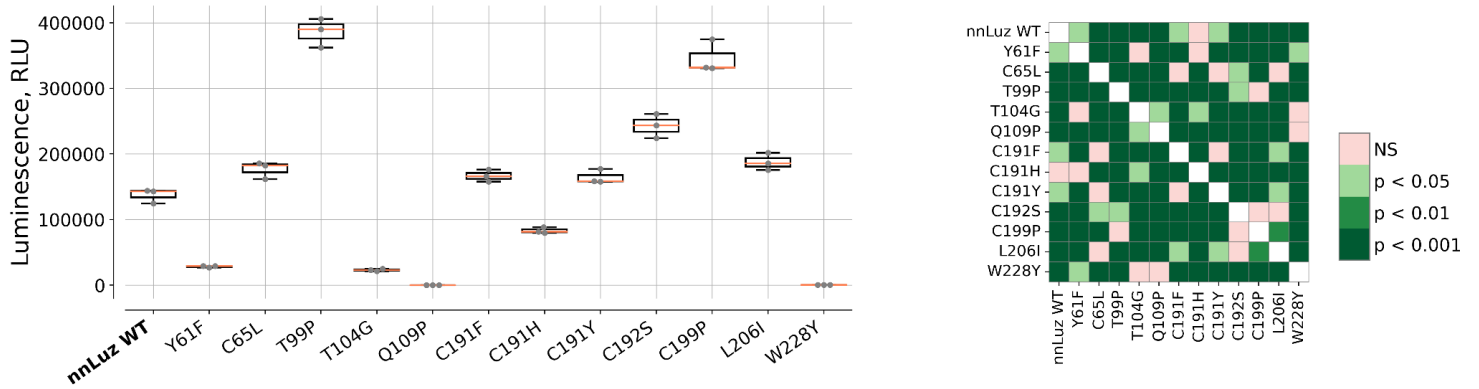




An improved pathway for autonomous bioluminescence imaging in eukaryotes

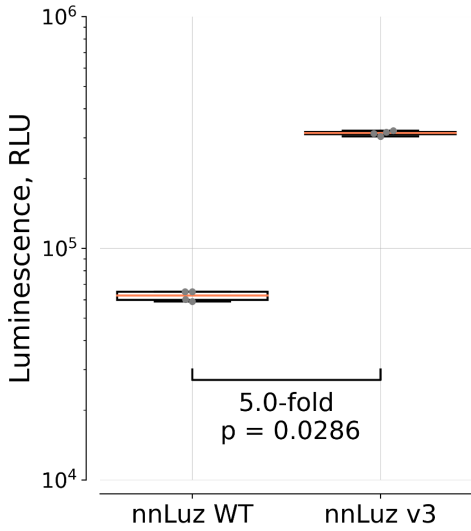
In the format provided by the authors and unedited

Escherichia coli, lysates,
after 100 μ M luciferin treatment

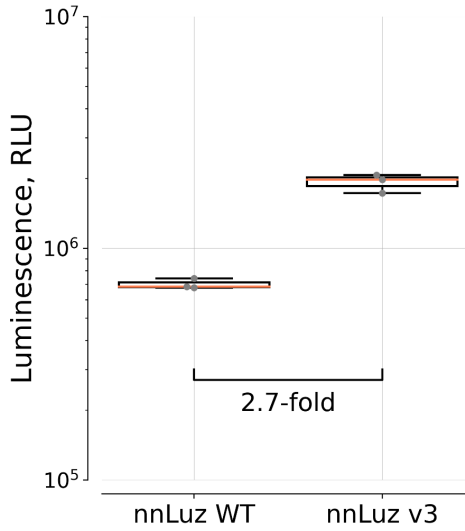


Supplementary Figure 1. Activity of nnLuz mutants obtained in consensus mutagenesis in *E. coli* lysates, after treatment with 100 μ M of luciferin. Imaging was performed at room temperature. Maximal brightness of luminescence is shown. Box and whisker plots (left) are accompanied by colour-coded p-values of post-hoc two-sided Conover's test (right) corrected by the step-down method using Sidak adjustments. NS – non-significant. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. N = 3 biologically independent samples per box plot. Kruskal-Wallis H Test: H-statistic = 37.20, p = 2.1e-04.

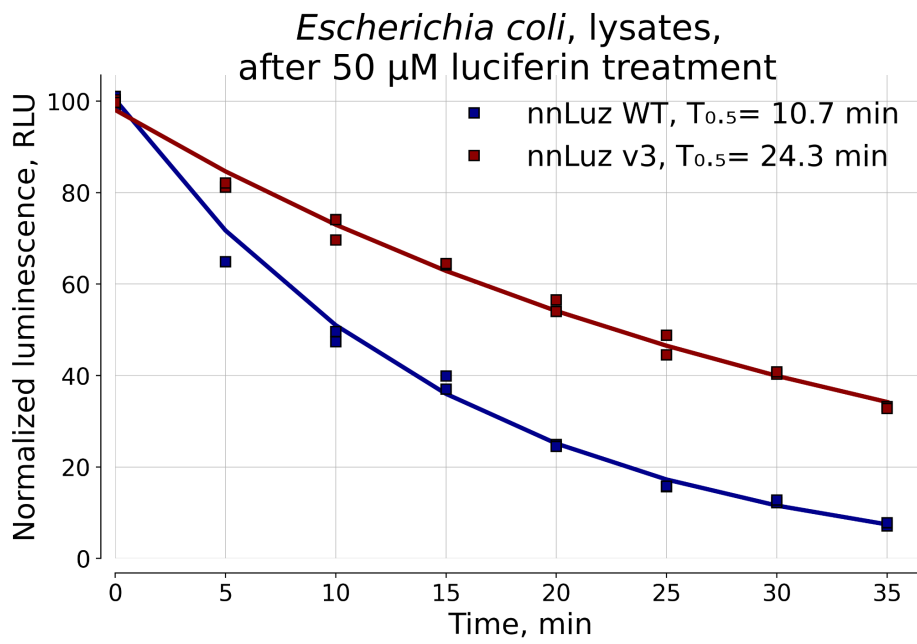
a *Escherichia coli*, lysates,
after 100 μ M luciferin treatment



b HEK293, transient expression,
after 740 μ M luciferin treatment



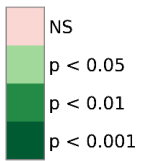
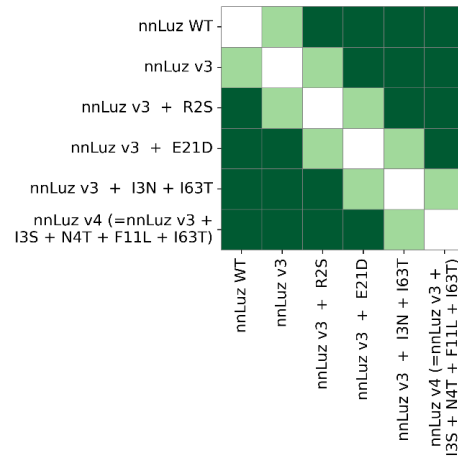
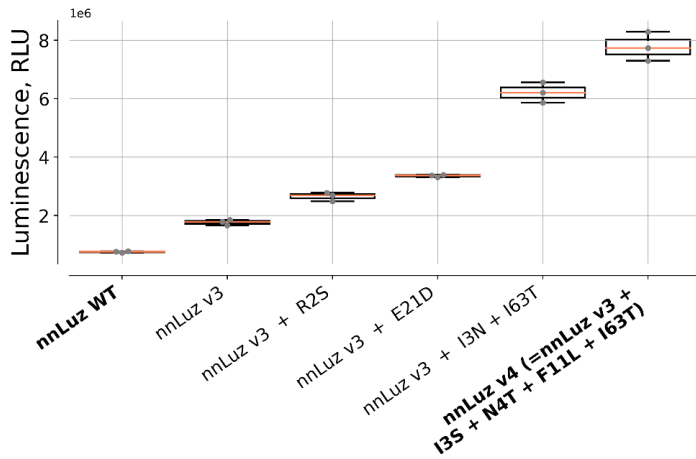
Supplementary Figure 2. Activity of nnLuz v3 (T99P, T192S, A199P) mutant in *E. coli* lysates (**a**) and in transient expression in HEK293 cells (**b**) after treatment with 100 or 740 μ M luciferin, respectively. Experiments in *E. coli* lysates were performed at room temperature, in mammalian cells – at 37°C. Maximal brightness of luminescence is shown. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The difference between mean values and p-values of post-hoc two-sided Mann-Whitney U-tests (if applicable) are indicated below the brackets between the box plots. N = 4 (**a**) or 3 (**b**) biologically independent samples per box plot.



Supplementary Figure 3. Activity of nnLuz WT and nnLuz v3 (T99P, T192S, A199P) in *E. coli* cleared lysates after incubation at 22°C during different periods of time followed by treatment with luciferin (50 μ M). Data points are normalised to the data at the time = 0 min. Data is shown as data points (squares) and fitting curves (lines). Data is fitted with an exponential decay equation. N = 2 biologically independent samples.

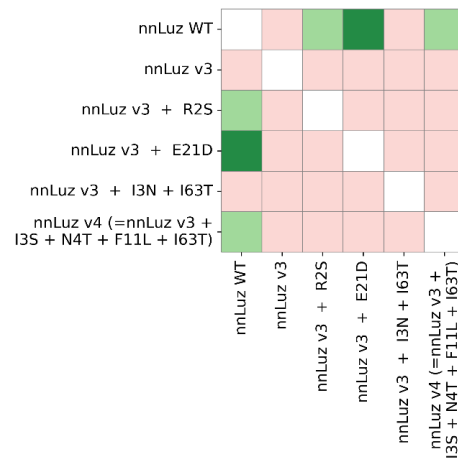
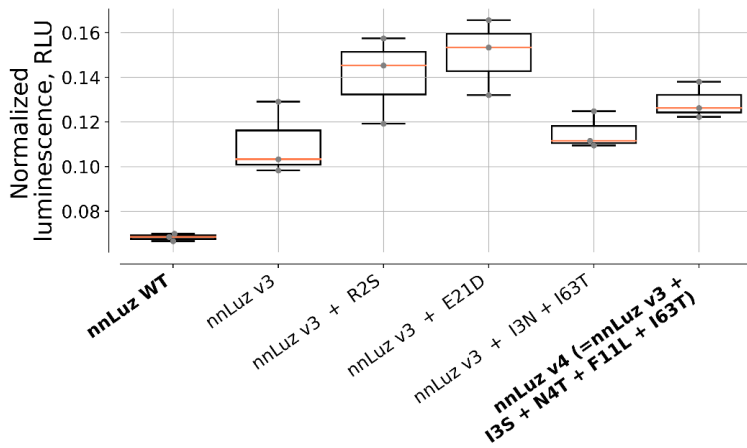
Escherichia coli, lysates,
after 100 μ M luciferin treatment

a



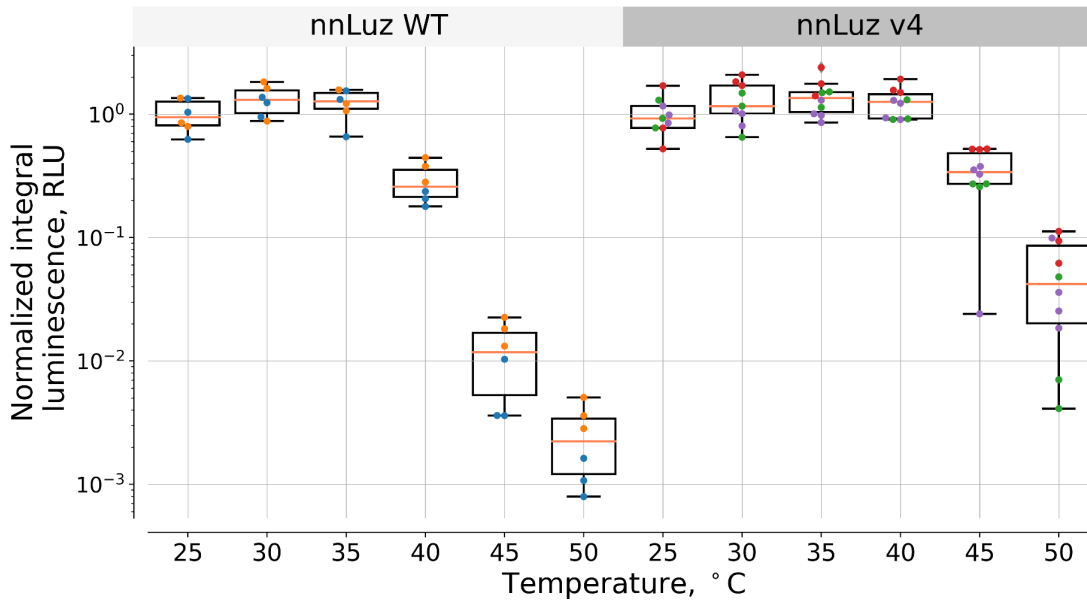
HEK293, transient expression,
after 740 μ M luciferin treatment

b



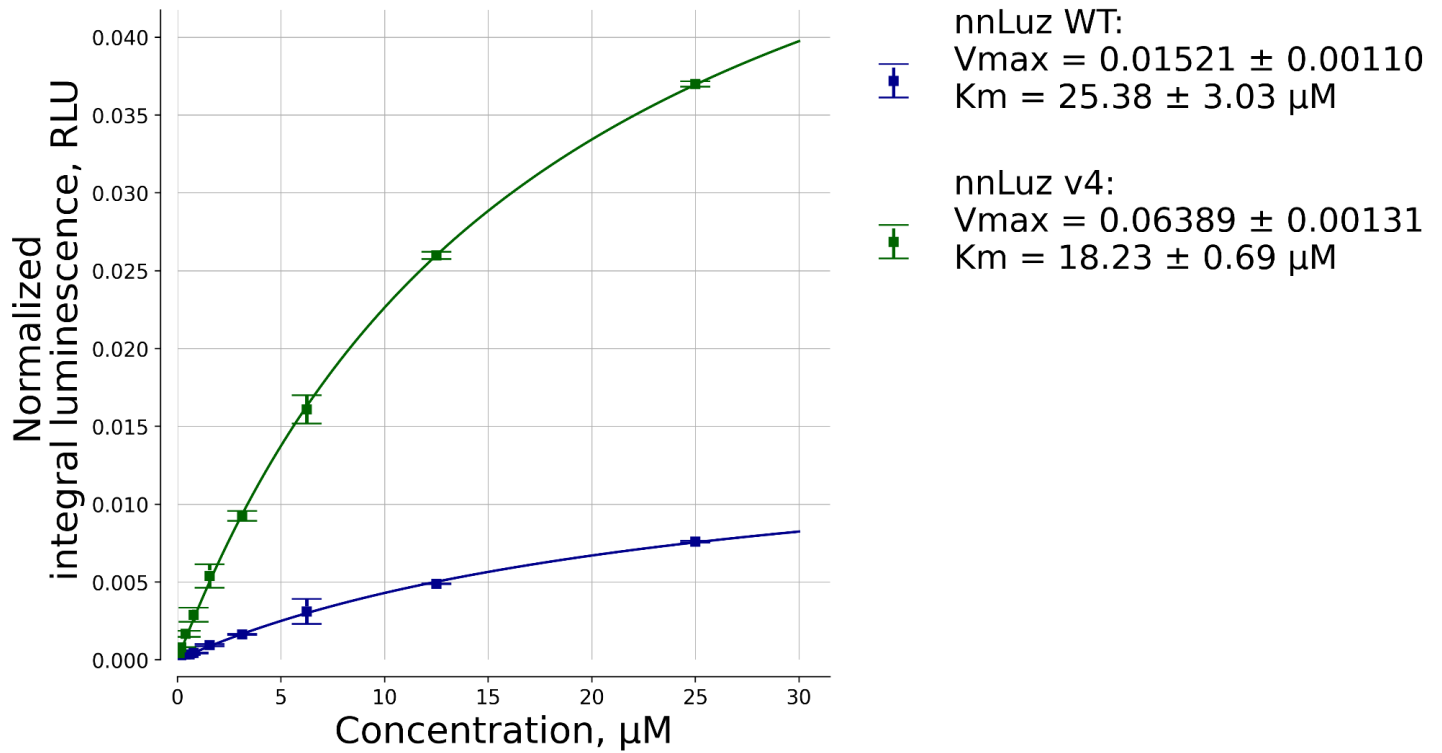
Supplementary Figure 4. Activity of mutants obtained after random mutagenesis of nnLuz v3 in *E. coli* lysates (a) and in transient expression in HEK293 cells (b) after treatment with luciferin. Maximal brightness of luminescence is shown. In HEK293 luminescence signal was normalised to protein abundance, as measured by HiBiT assay. Box and whisker plots (left) are accompanied by colour-coded p-values of post-hoc two-sided Conover's test (right) corrected by the step-down method using Sidak adjustments. NS – non-significant. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. N = 3 biologically independent samples per box plot. Kruskal-Wallis H Test: H-statistic = 16.58, p = 5.4e-03 (a) and H-statistic = 12.84, p = 2.5e-02 (b).

Pichia pastoris, stable, after 50 μ M luciferin treatment



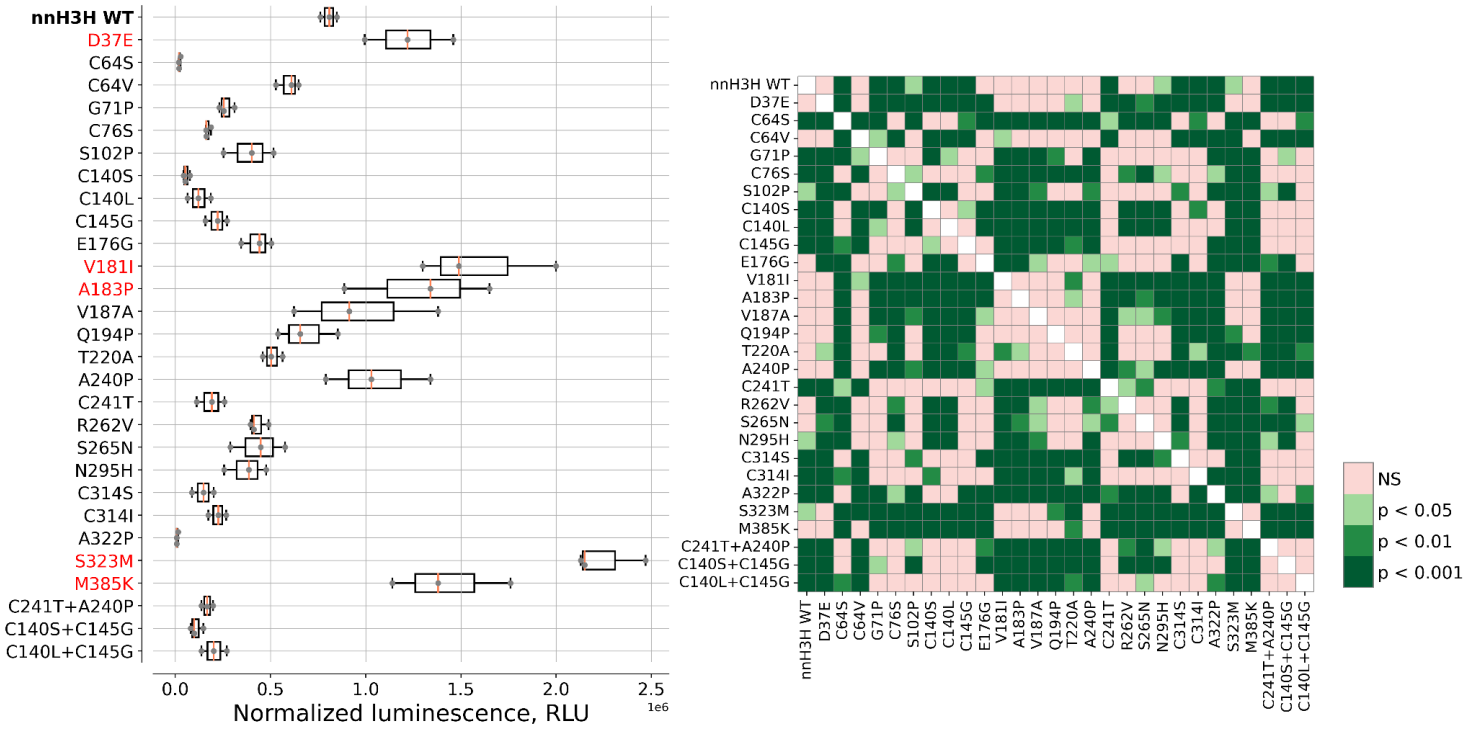
Supplementary Figure 5. Luminescence of yeast cells expressing luciferase variants, after 10 min incubation at different temperatures followed by treatment with luciferin (50 μ M). The integral luminescence for 10 min normalised to the integral signal obtained after incubation at 25°C is shown. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different yeast strains (2 strains for nnLuz WT and 3 strains for nnLuz v4). N = 6-10 biologically independent samples per box plot.

Pichia pastoris, lysates



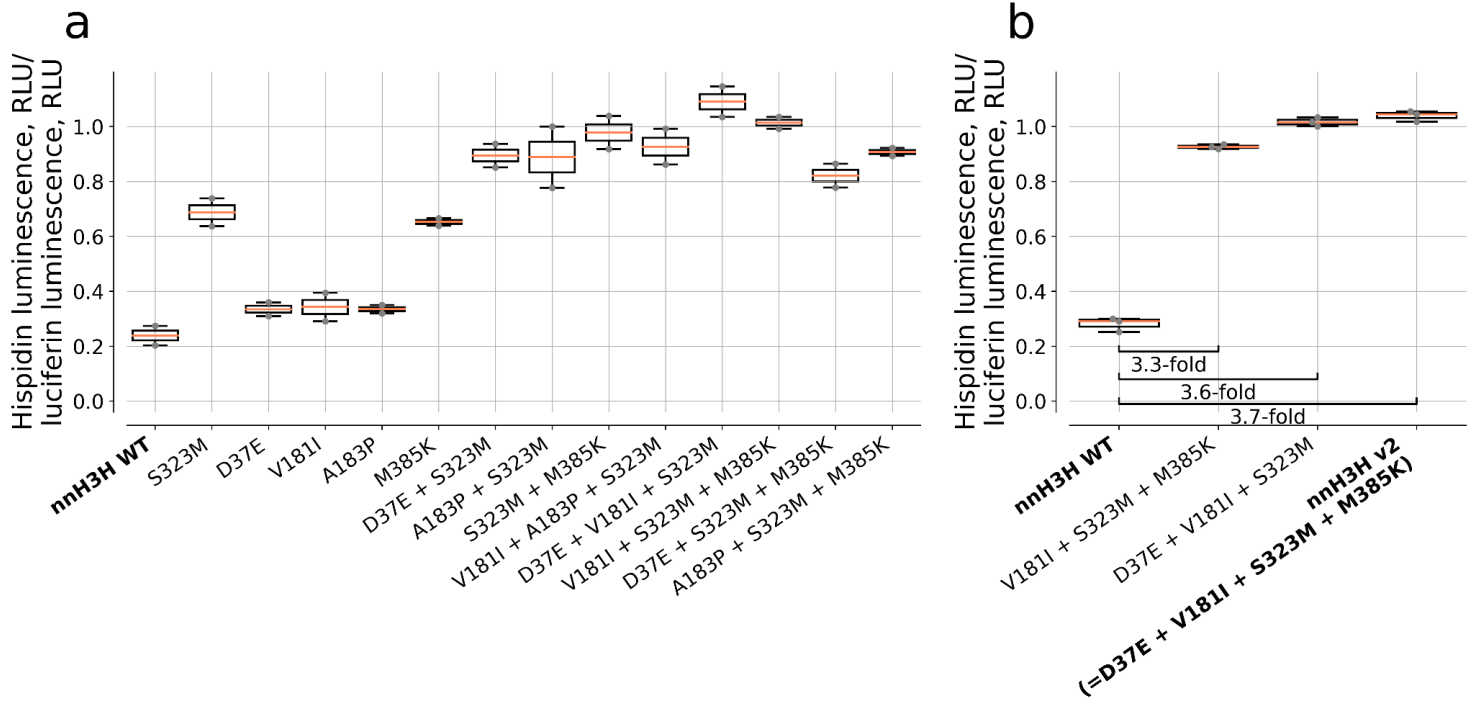
Supplementary Figure 6. Michaelis–Menten curve of nnLuz WT/v4–HiBIT fusions obtained from yeast lysates in the presence of 0.1 - 25 μM fungal luciferin. Data normalized to expression level assessed by HiBit luminescence. Data is shown as data points (squares) \pm SD (whiskers) and fitting curves (lines). Data is fitted with a Michaelis-Menten equation. N = 3 biologically independent samples per box plot.

HEK293, transient expression,
after 0.8 mM hispidin treatment

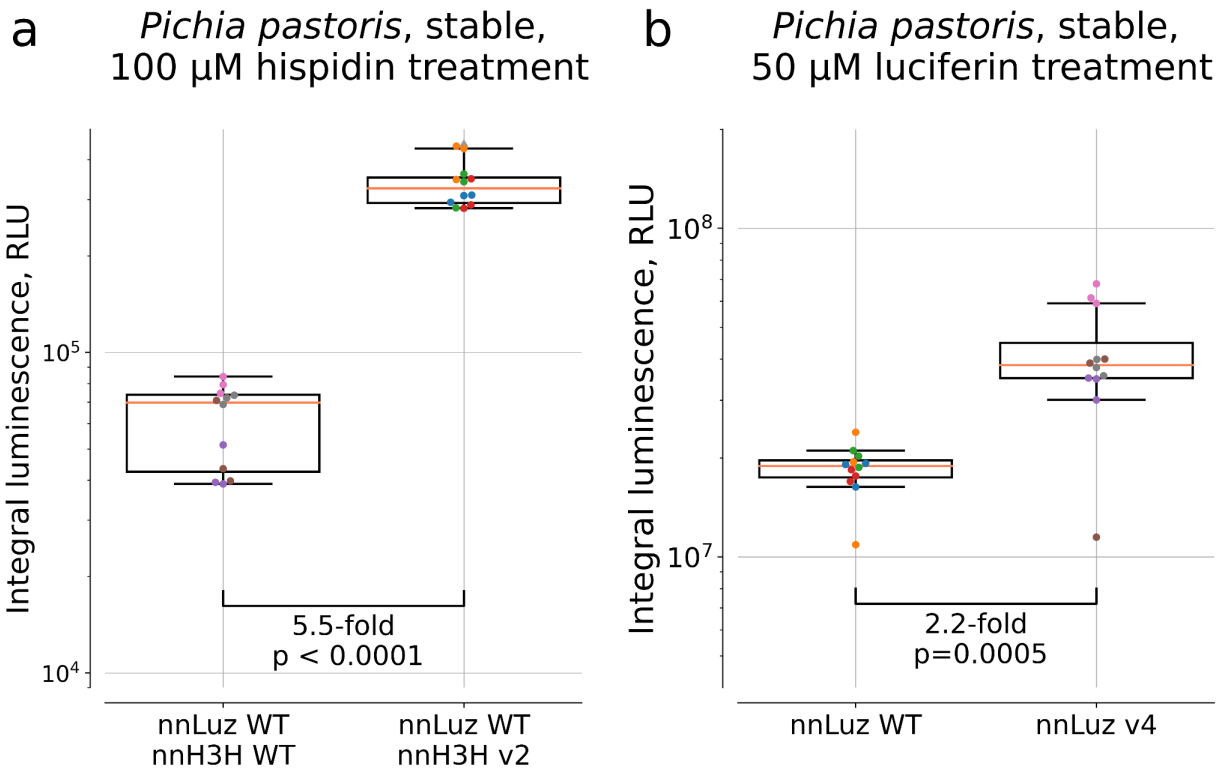


Supplementary Figure 7. Activity of mutants obtained in consensus mutagenesis of nnH3H. Transient co-expression of depicted nnH3H mutants with nnLuz v3 in HEK293 cells, luminescence measured after addition of 0.8 mM hispidin. Luminescence signal was normalised to protein abundance, as measured by HiBiT assay. Red labels correspond to mutations with improved luminescence. Box and whisker plots (left) are accompanied by colour-coded p-values of post-hoc two-sided Conover's test (right) corrected by the step-down method using Sidak adjustments. NS – non-significant. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. N = 3 biologically independent samples per box plot. Kruskal-Wallis H Test: H-statistic = 82.22, $p = 3.1e-07$.

HEK293, transient expression,
after 0.8 mM hispidin or luciferin treatment



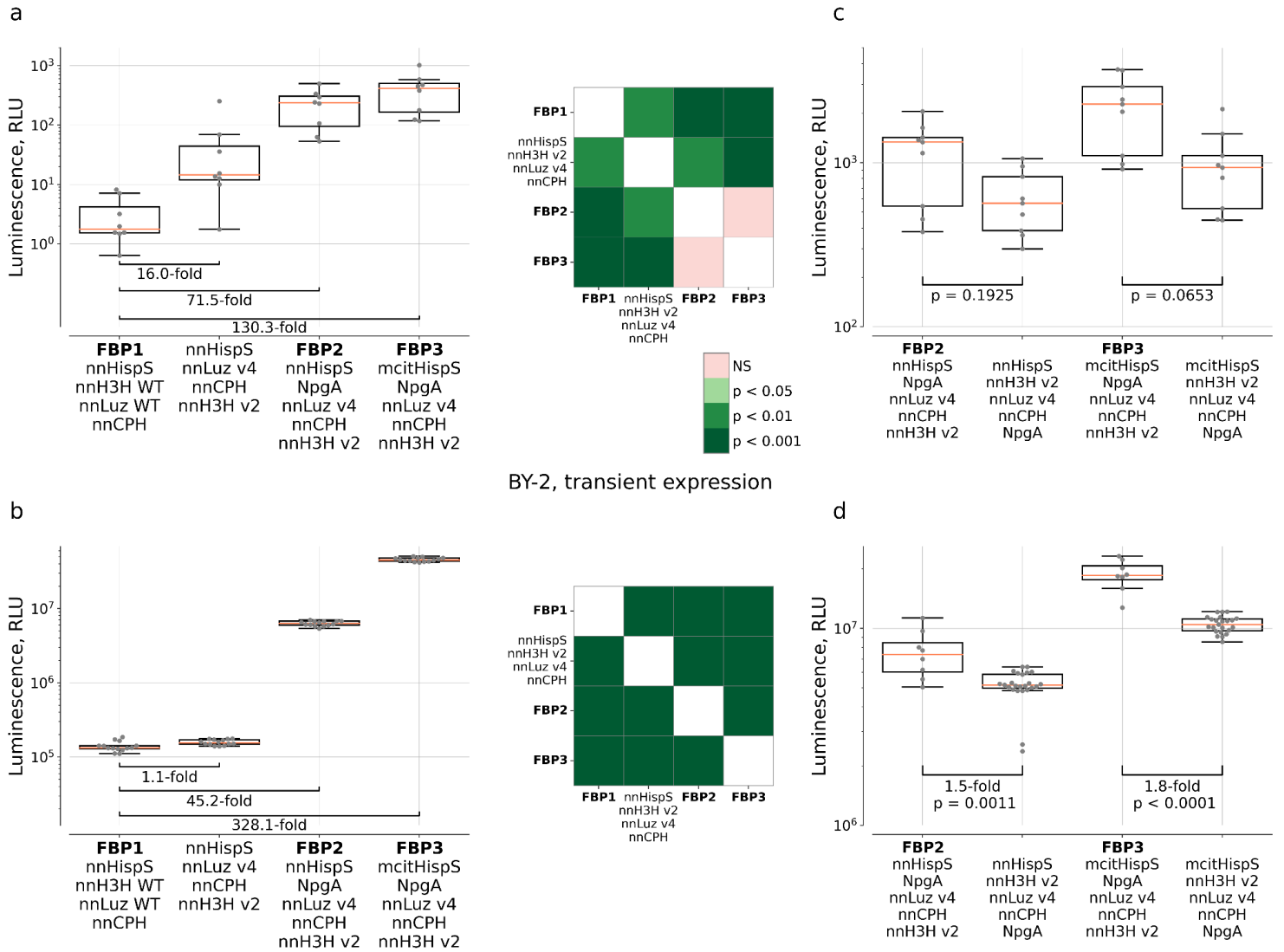
Supplementary Figure 8. Activity of nnH3H mutants in transient expression with nnLuz v3 in HEK293 after treatment with 0.8 mM of luciferin or hispidin. **a.** Combinatorial mutation analysis of nnH3H. N = 2 biologically independent samples per box plot. **b.** Follow-up experiment to assess selected combinations of mutations. N = 3 biologically independent samples per box plot. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median.



Supplementary Figure 9. Comparison of WT and improved enzymes of nnH3H and nnLuz in *P. pastoris*.

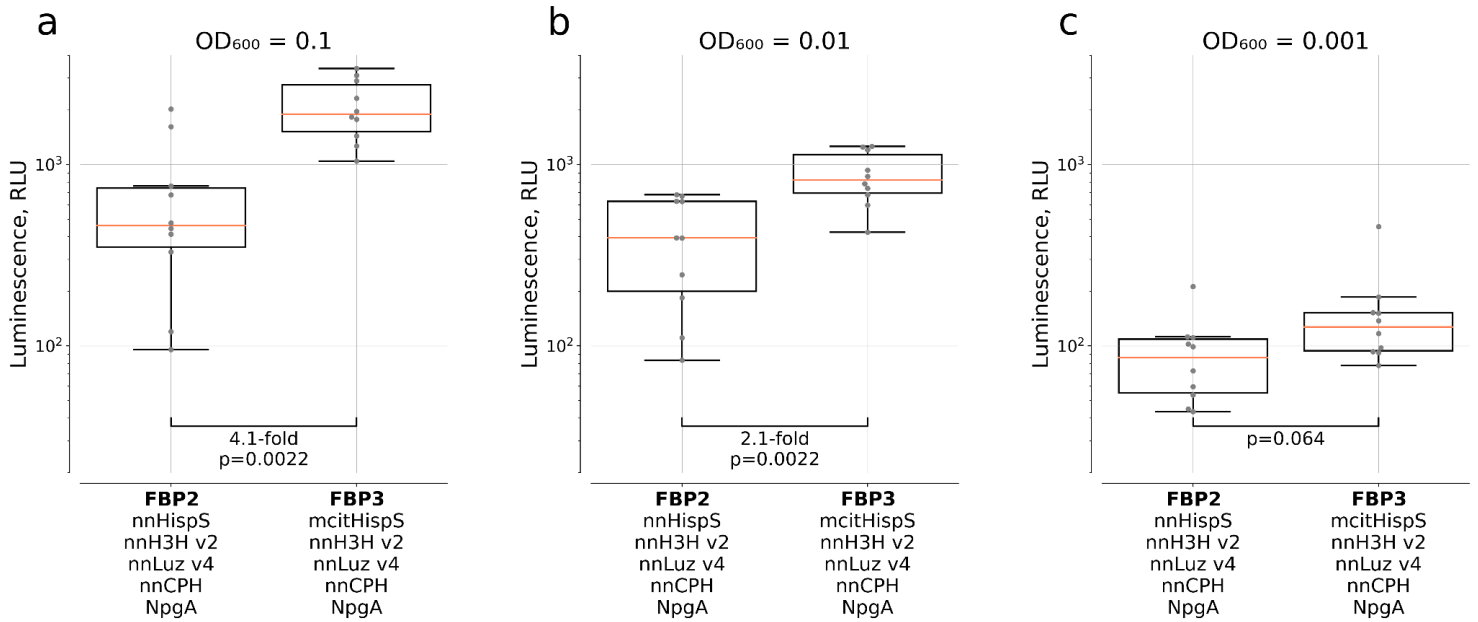
Luminescence was assayed after adding 100 μ M hispidin (a) and 50 μ M luciferin (b). Integral signal was collected for 20 (a) and 10 min (b). The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different yeast strains (4 per box plot). N = 12 biologically independent samples per box plot. The difference between mean values and p-values of post-hoc two-sided Mann-Whitney U-tests are indicated below the brackets between the box plots, p = 3.7e-05 (a), p = 4.8e-04 (b).

Nicotiana benthamiana, transient expression



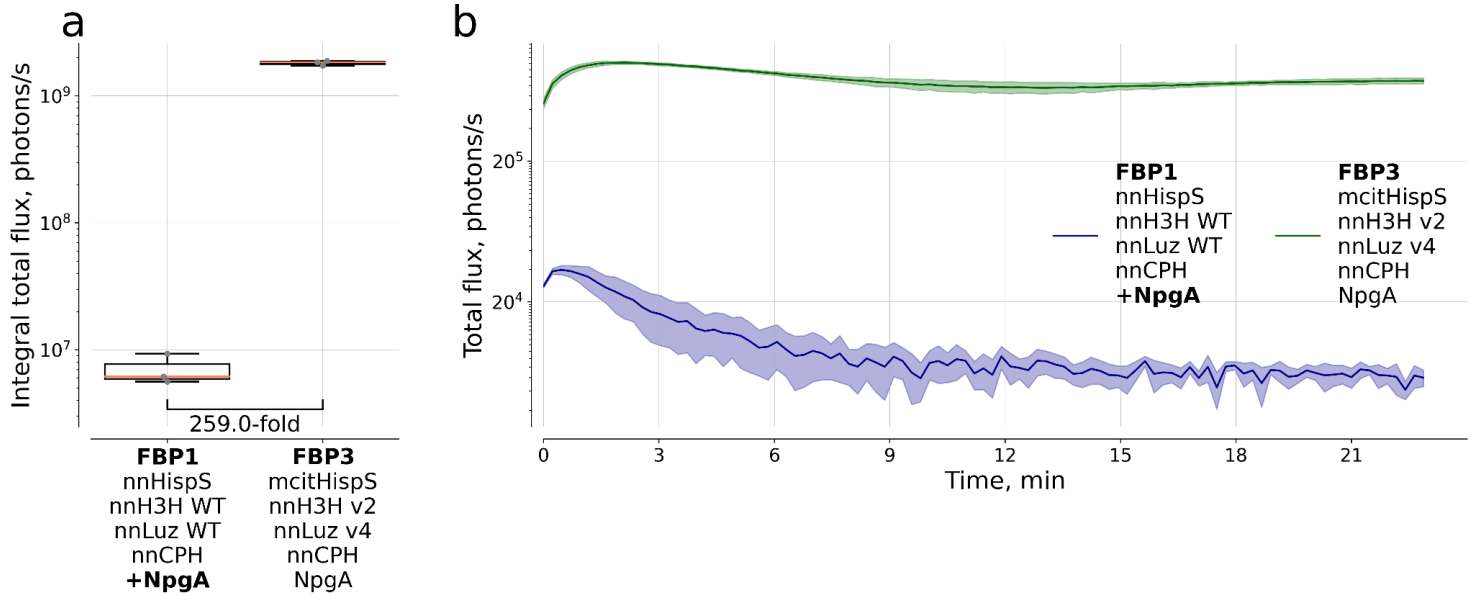
Supplementary Figure 10. Comparison of FBP versions, and assembly variants with different order of transcription units. The experiments were performed in *N.benthamiana* leaves (a, c) and BY-2 plant cell packs (b, d). Named versions refer to plasmids shown on Extended Data Figure 4 (a, b). The order of gene names in the labels of the X axis corresponds to the order of transcription units in multi-gene plasmids. Box and whisker plots are accompanied by colour-coded p-values of two-sided Conover's test corrected by the step-down method using Sidak adjustments (a, b). NS – non-significant. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. N = 8 (a) or 9 (c) leaves per box plot, N = 14 (b) or 8-24 (d) cell packs per box plot. The difference between mean values is indicated below the brackets between the box plots, in (c, d) supplied with p-value of post-hoc two-sided Mann-Whitney U-test. In (d) p = 1.1e-03 and 2.5e-07 for FBP2 vs nnHispS + nnH3H v2 + nnLuz v4 + nnCPH + NpgA and FBP3 vs mcitHispS + nnH3H v2 + nnLuz v4 + nnCPH + NpgA, respectively. Kruskal-Wallis H Test: H-statistic = 23.59, p = 3.0e-5 (a) and H-statistic = 48.10, p = 2.0e-10 (b).

Nicotiana benthamiana, transient expression

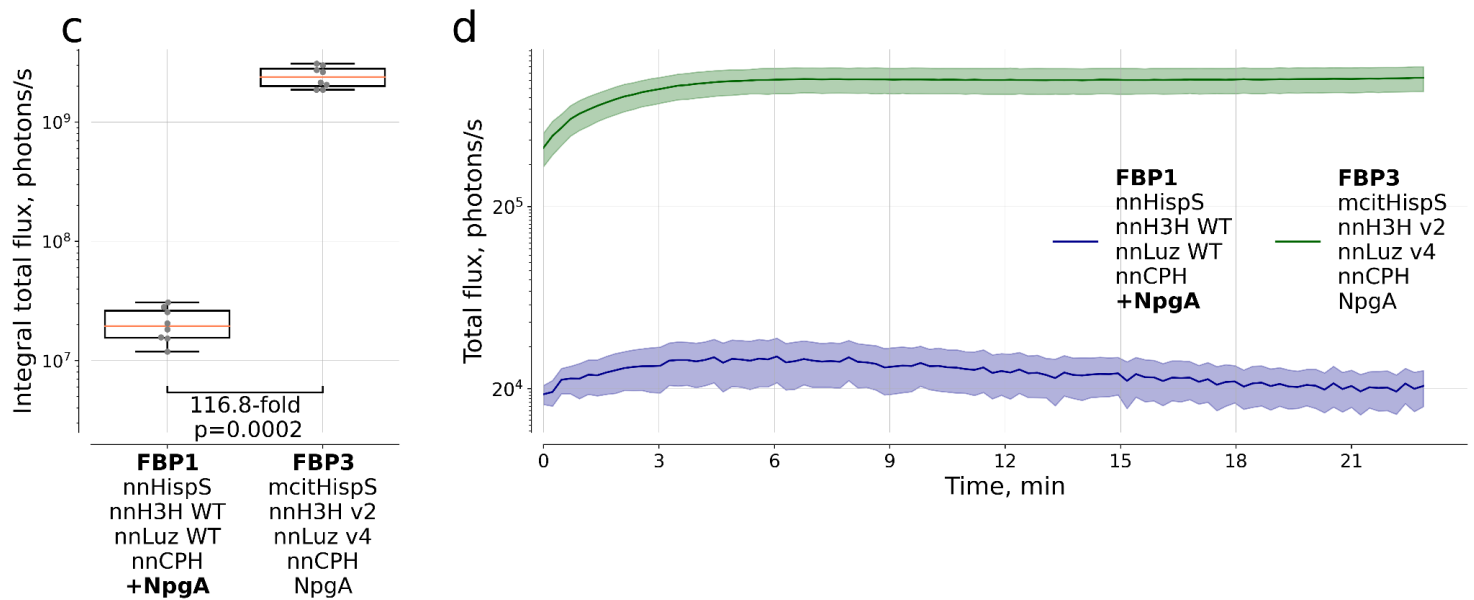


Supplementary Figure 11. Comparison of FBP2 and FBP3 pathways in *N. benthamiana* leaves infiltrated with agrobacterial suspensions of different optical densities. $OD_{600} = 0.1$ (a), 0.01 (b) or 0.001 (c). The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. $N = 10$ leaves per box plot. The difference between mean values and p-values of post-hoc two-sided Mann-Whitney U-tests are indicated below the brackets between the box plots.

HEK293NT, transient expression, after 25 μ M caffeic acid treatment

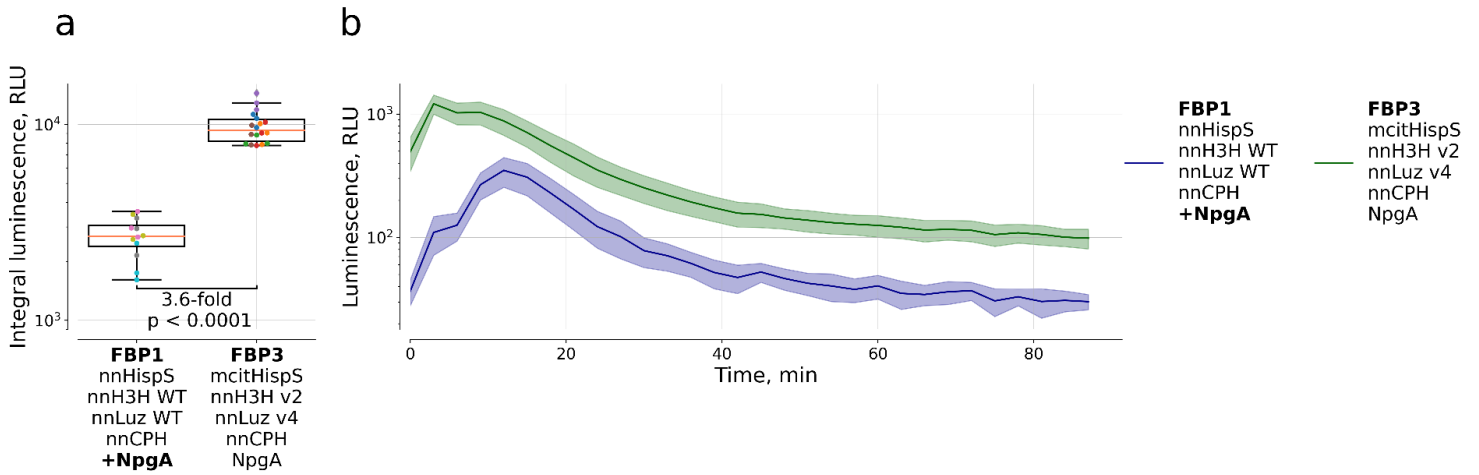


HEK293NT, transient expression, after 100 μ M caffeic acid treatment

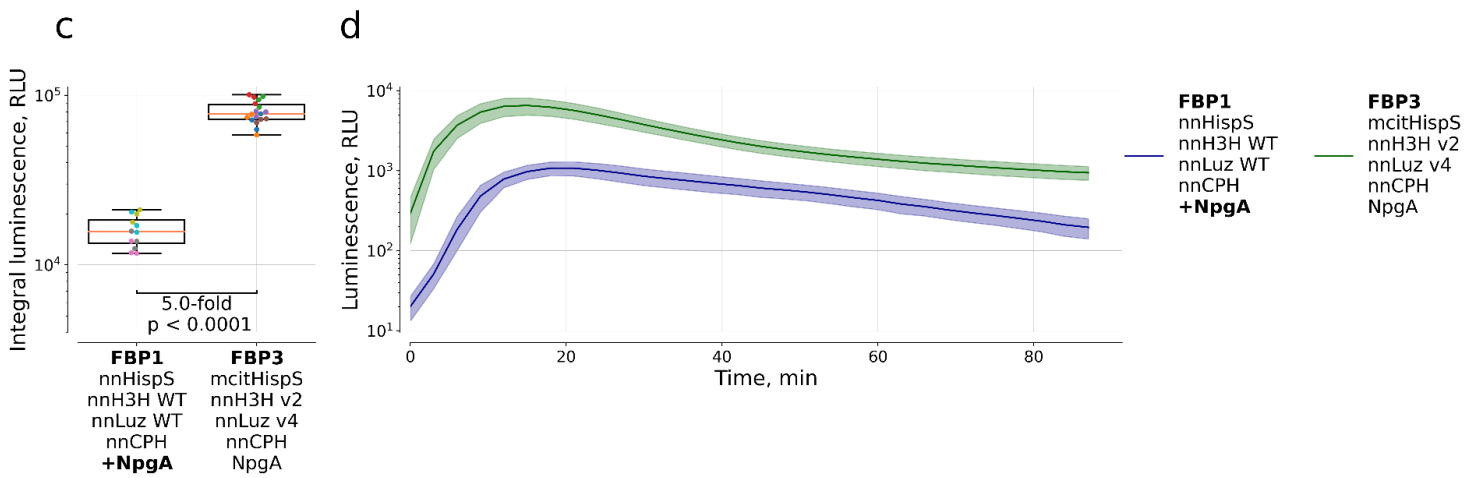


Supplementary Figure 12. FBP1 and FBP3 pathways comparison in transient expression assay in HEK293NT cells after 25 μ M (a,b) and 100 μ M (c, d) caffeic acid treatment. (a, c) Integral signal for 23 minutes. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. N = 3 (a) and 8 (c) biologically independent replicates per box plot. The difference between mean values and p-values of post-hoc two-sided Mann-Whitney U-tests (if applicable) are indicated below the brackets between the box plots. (b,d) Kinetics. Data shown as mean (solid line) \pm SD (area around the solid line).

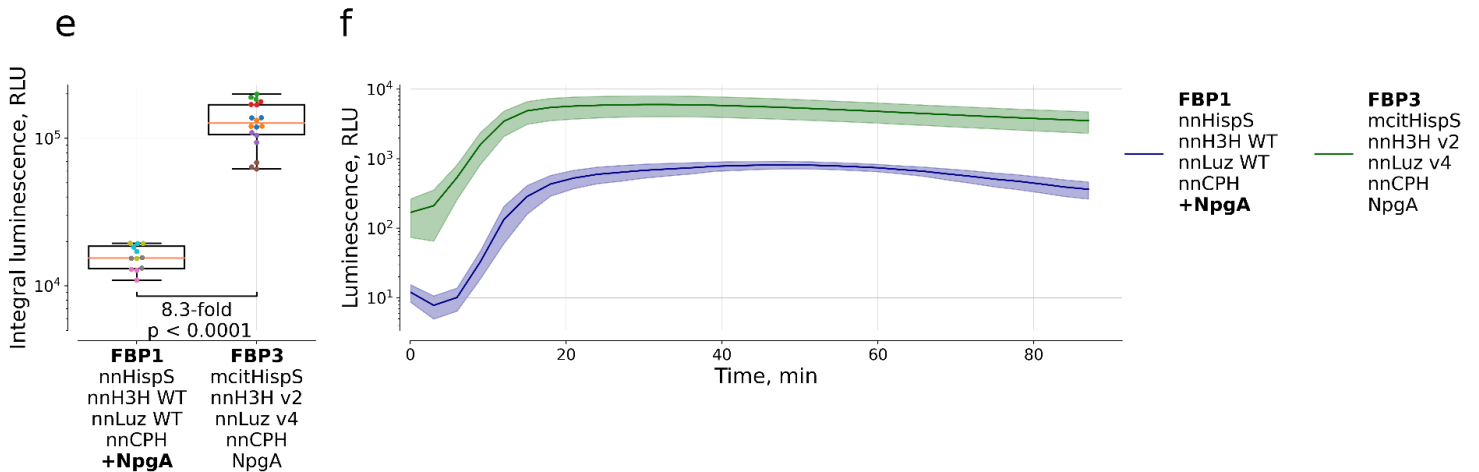
Pichia pastoris, stable, after 100 μ M caffeic acid treatment



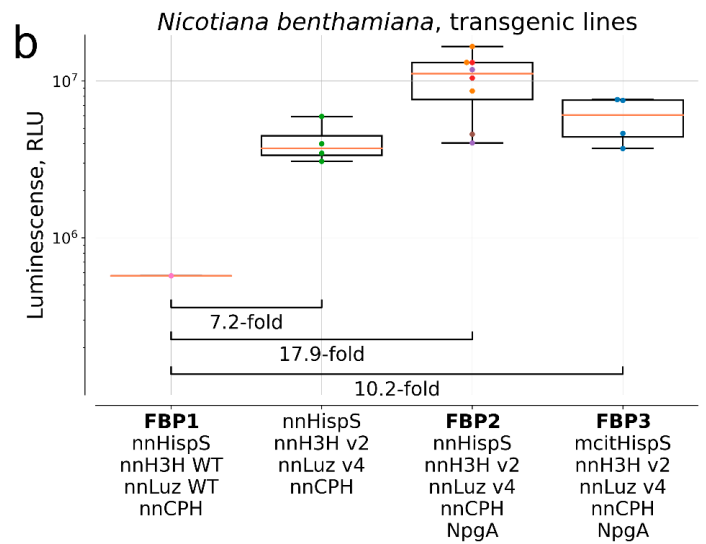
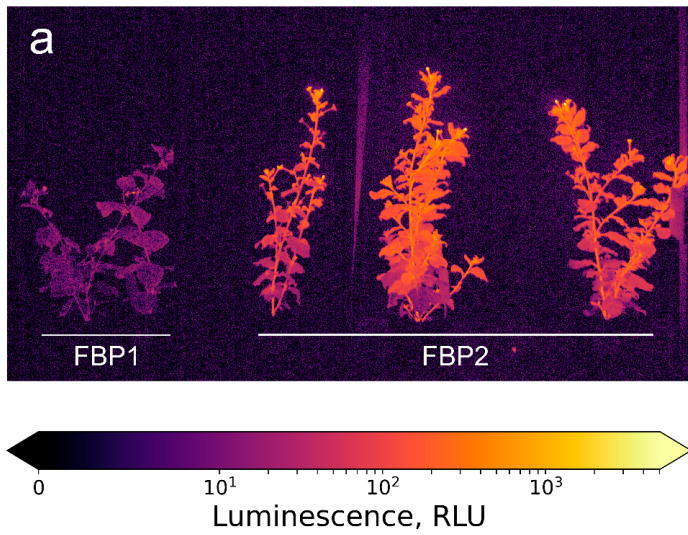
Pichia pastoris, stable, after 100 mM caffeic acid treatment



Pichia pastoris, stable, after 220 mM caffeic acid treatment

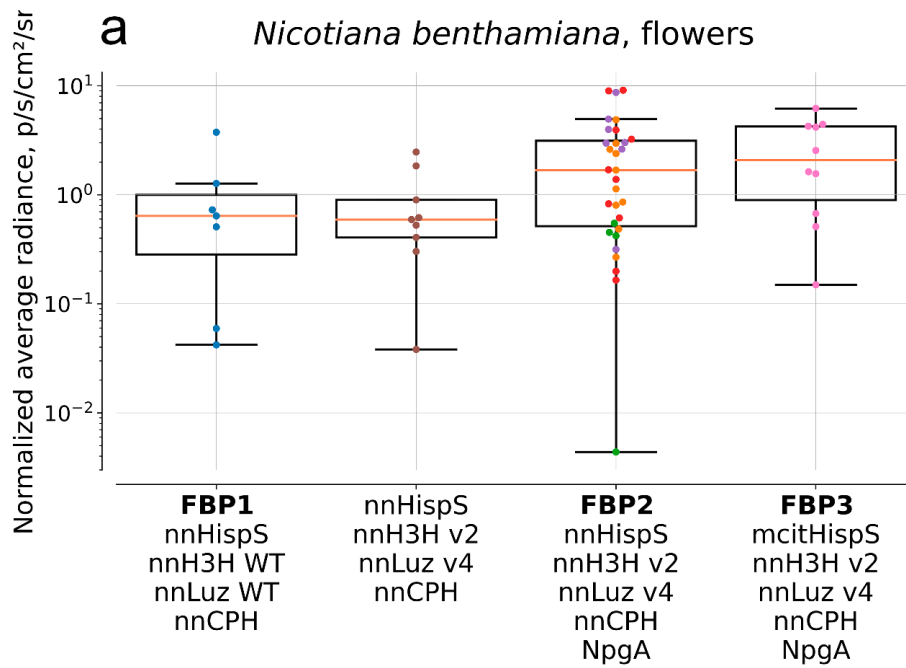


Supplementary Figure 13. The comparison of *P. pastoris* strains expressing FBP1 or FBP3 pathways after 100 μ M (a, b), 100 mM (c, d) and 220 mM (e, f) caffeic acid treatment. (a, c, e) Integral signal for 90 minutes. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different yeast strains (4 yeast strains for FBP1 and 6 for FBP3). The difference between mean values and p-values of post-hoc two-sided Mann-Whitney U-tests are indicated below the brackets between the box plots, $p = 5.0 \times 10^{-6}$ (a), $p = 5.0 \times 10^{-6}$ (c), $p = 5.0 \times 10^{-6}$ (e). $N = 12$ or 18 biologically independent replicates for FBP1 or FBP3 in each panel, respectively. (b, d, f) Kinetics. Data shown as mean (solid line) \pm SD (area around the solid line).

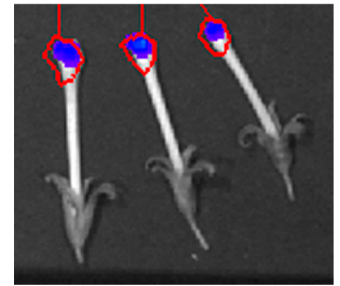


Supplementary Figure 14. Imaging of 9-week-old transgenic *N. benthamiana*. The FBP1 is the plant line NB021 reported in ref ¹. The photo, captured with ISO 400 and exposure of 30 sec (a) and integral luminescent signal from whole plants (b). It is interesting to note that when FBP1 is not co-expressed with NpgA, *N. benthamiana* plants generally produce significantly more light than *N. tabacum* plants. We interpret this as evidence of higher levels of endogenous PPTase activity in *N. benthamiana*.

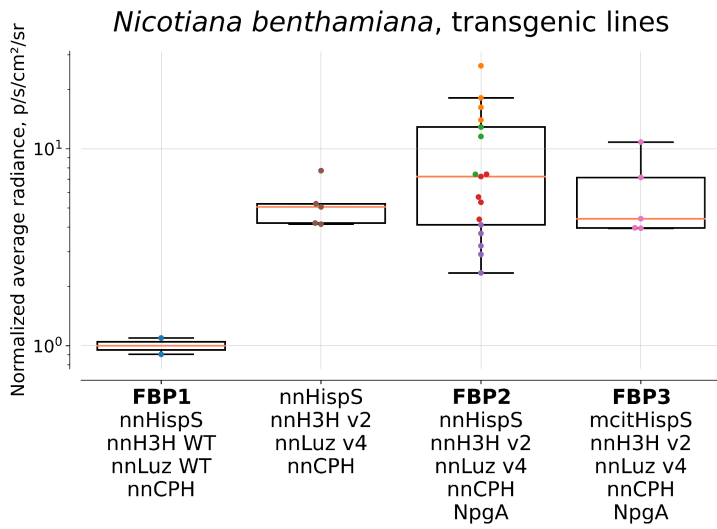
The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different plant lines: 1 line for FBP1 (N = 1 plant), 1 line for nnHisps + nnH3H v2 + nnLuz v4 + nnCPH (N = 4 plants), 4 lines for FBP2 (N = 8 plants), 1 line for FBP3 (N = 4 plants). The difference between mean values is indicated below the brackets between the box plots.



b

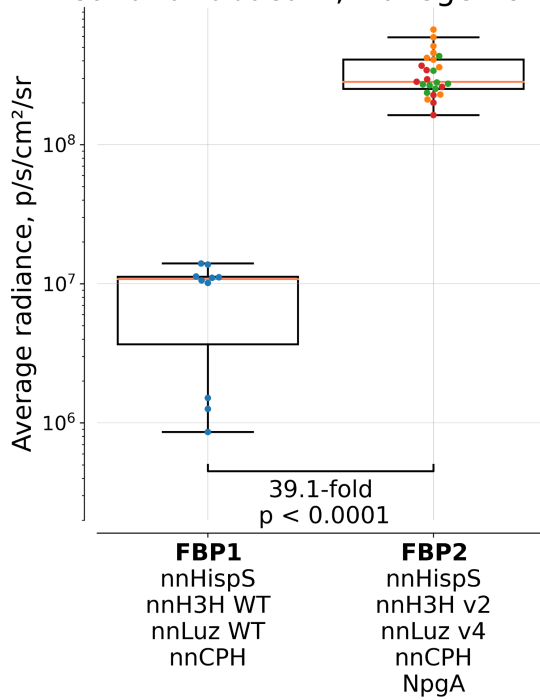


Supplementary Figure 15. Imaging of flowers of 7-week-old transgenic *N. benthamiana*. The FBP1 is the plant line NB021 reported in ref¹. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different plant lines: NB021 line for FBP1 (N = 7 flowers), 1 line for nnHisps + nnH3H v2 + nnLuz v4 + nnCPH (N = 9 flowers), 4 lines for FBP2 (N = 31 flowers), 1 line for FBP3 (N = 10 flowers). Kruskal-Wallis H Test: H-statistic = 6.48, p = 0.09. (c) Example of selected ROI of corona of flowers.

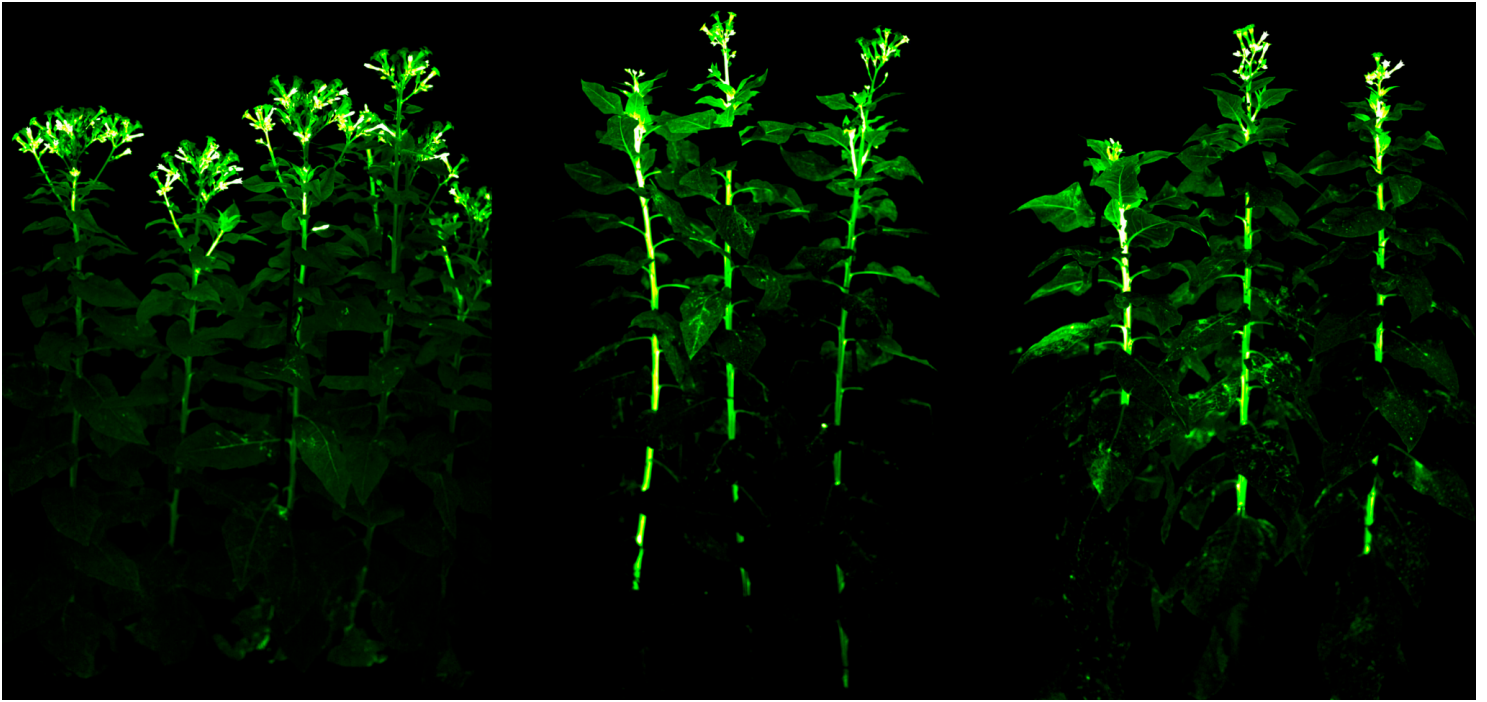


Supplementary Figure 16. Imaging of leaves taken from the top of 7-week-old transgenic *N. benthamiana*. The FBP1 is the plant line NB021 reported in ref¹. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different plant lines: NB021 line for FBP1 (N = 2 leaves), 1 line for nnHispS + nnH3H v2 + nnLuz v4 + nnCPH (N = 5 leaves), 4 lines for FBP2 (N = 17 leaves), 1 line for FBP3 (N = 5 leaves). Kruskal-Wallis H Test: H-statistic = 5.61, p = 0.13.

Nicotiana tabacum, transgenic lines



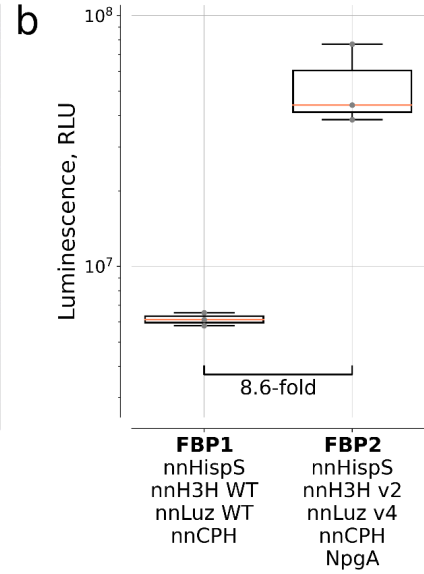
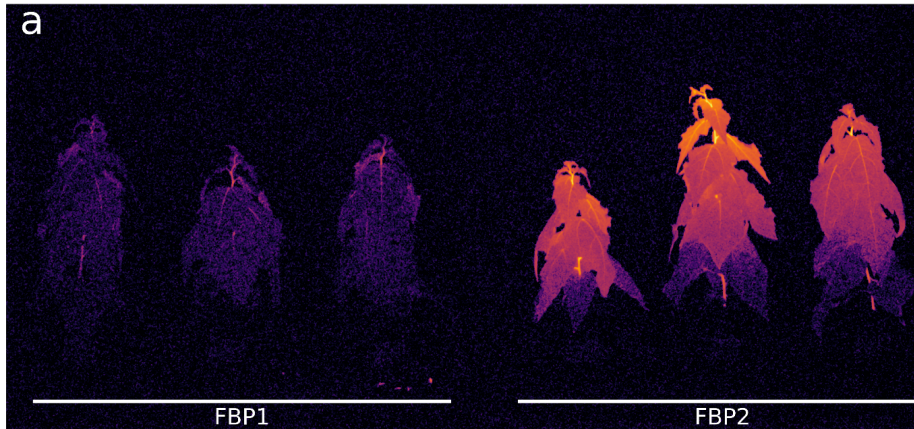
Supplementary Figure 17. Average luminescence of leaves taken from the top of 7.5-week-old *N. tabacum* expressing FBP1 or FBP2 pathways. The FBP1 is the plant line NT001 reported in ref¹. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different plant lines (FBP1 - NT001, for FBP2 - 3 lines per box plot); N = 10 or 25 leaves for FBP1 or FBP2, respectively. The difference between mean values and p-values of post-hoc two-sided Mann-Whitney U-test are indicated below the brackets between the box plots, p = 5.0e-06.



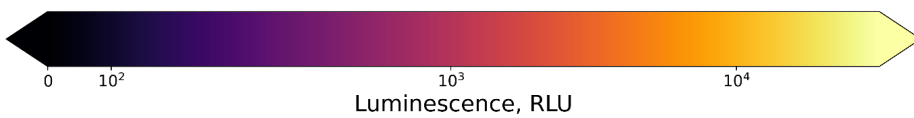
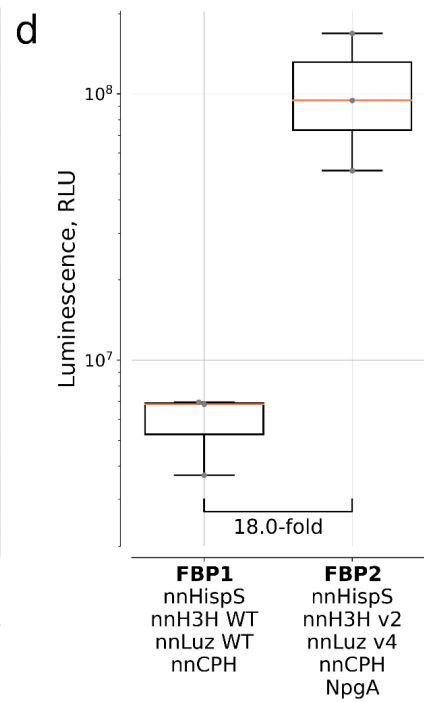
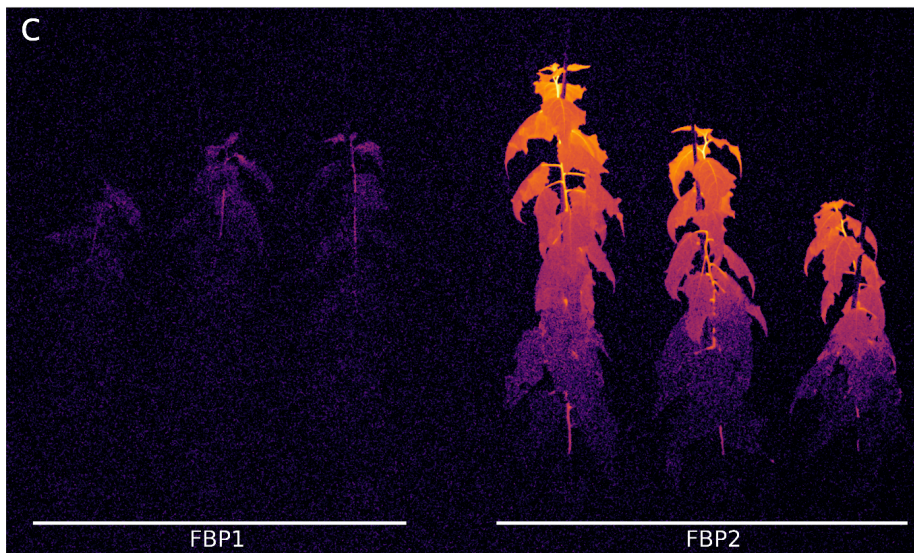
Supplementary Figure 18. Imaging of 9-week-old *N. tabacum* plants representing 3 different lines expressing FBP2 pathway. ISO 400, exposure 30 sec (see Methods).

Populus canadensis, transgenic lines

2-month-old

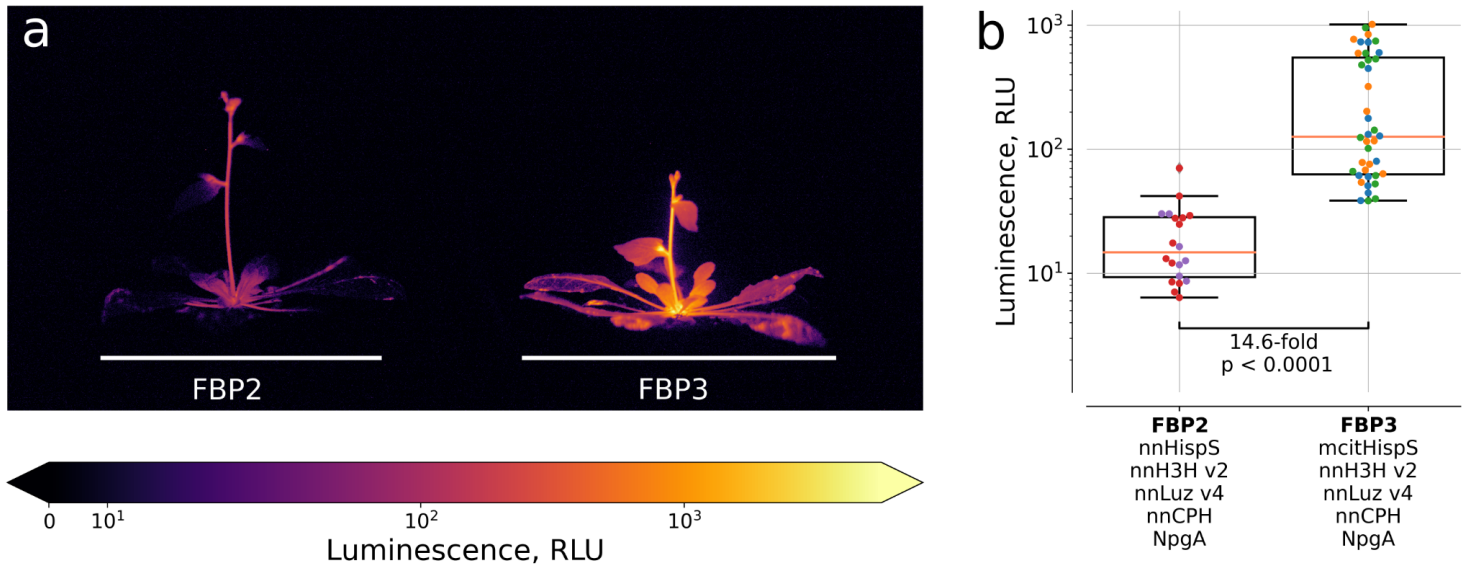


3-month-old

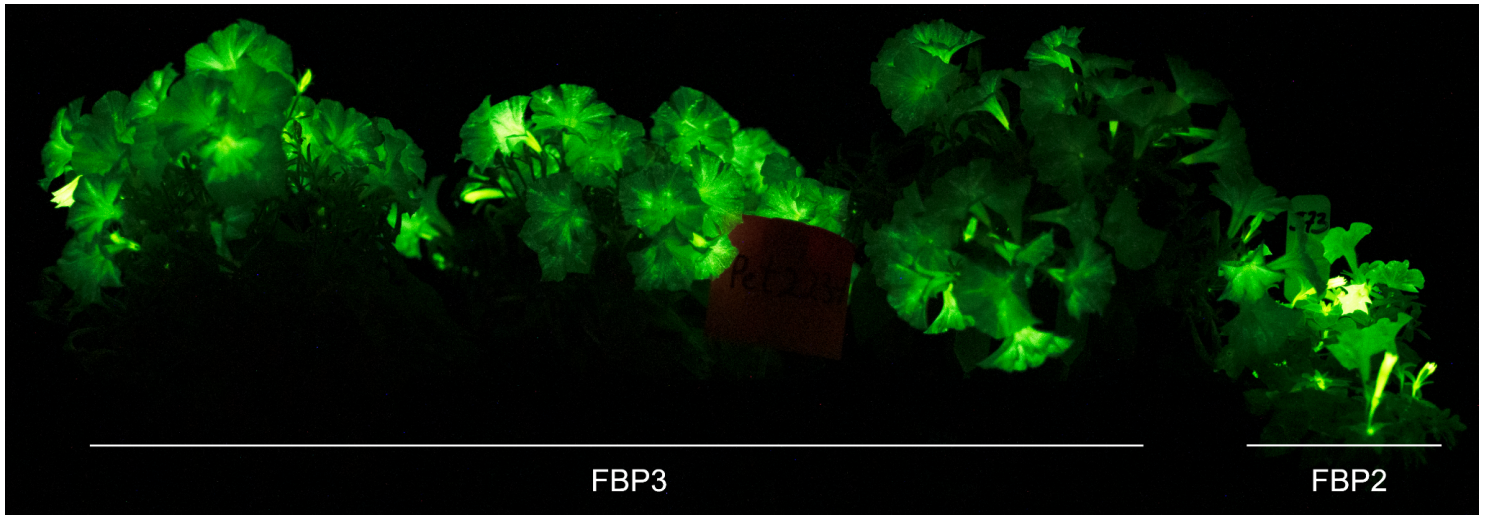


Supplementary Figure 19. Imaging of *P. canadensis* transformed with FBP1 or FBP2 pathways. The photo of 2-month-old (a) or 3-month-old plants (c) and corresponding integral luminescence signal from the whole plants (b, d). Photos were captured with ISO 20000 and exposure of 30 sec. The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. N = 3 plants per box plot. The difference between mean values is indicated below the brackets between the box plots.

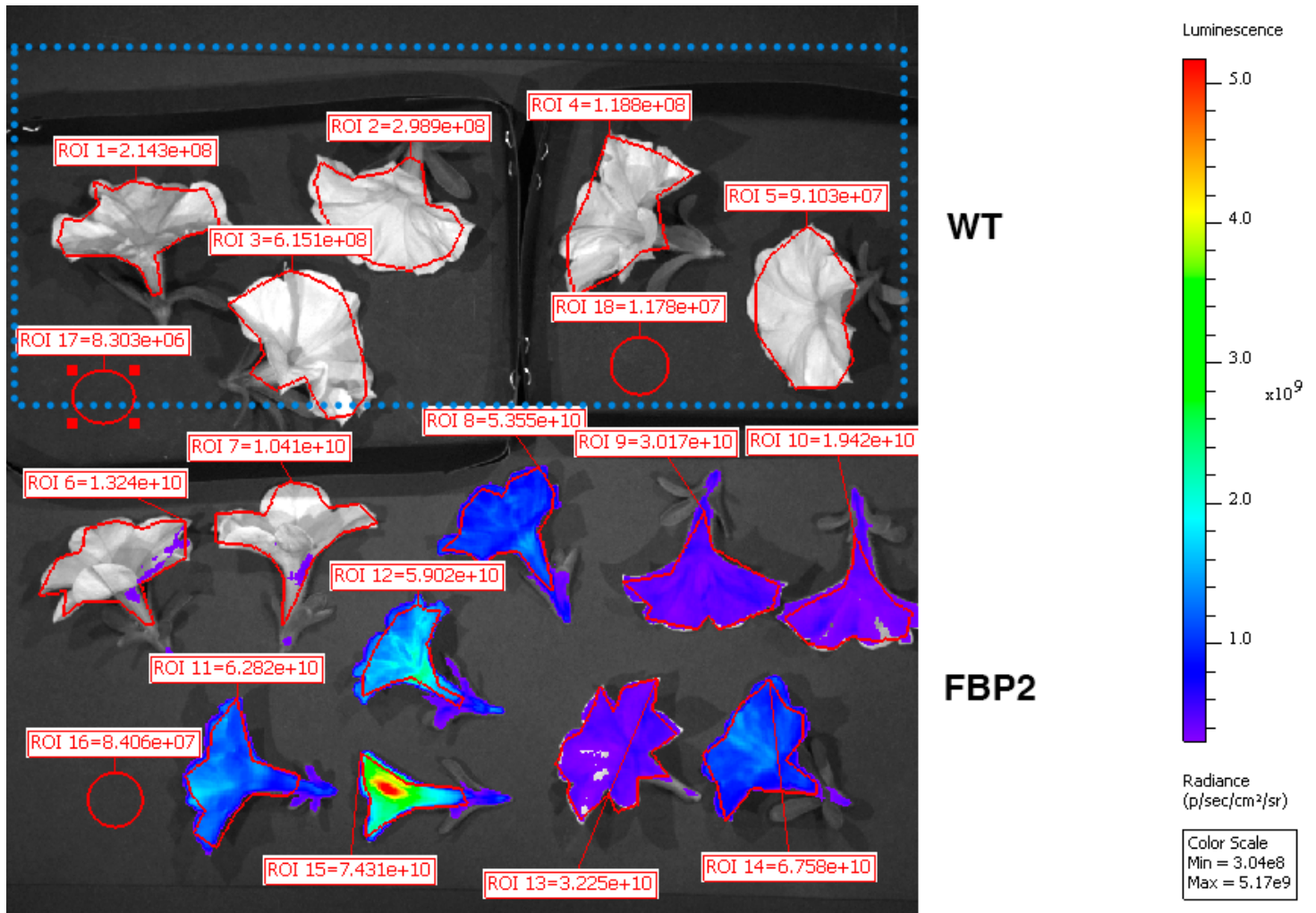
Arabidopsis thaliana, transgenic lines



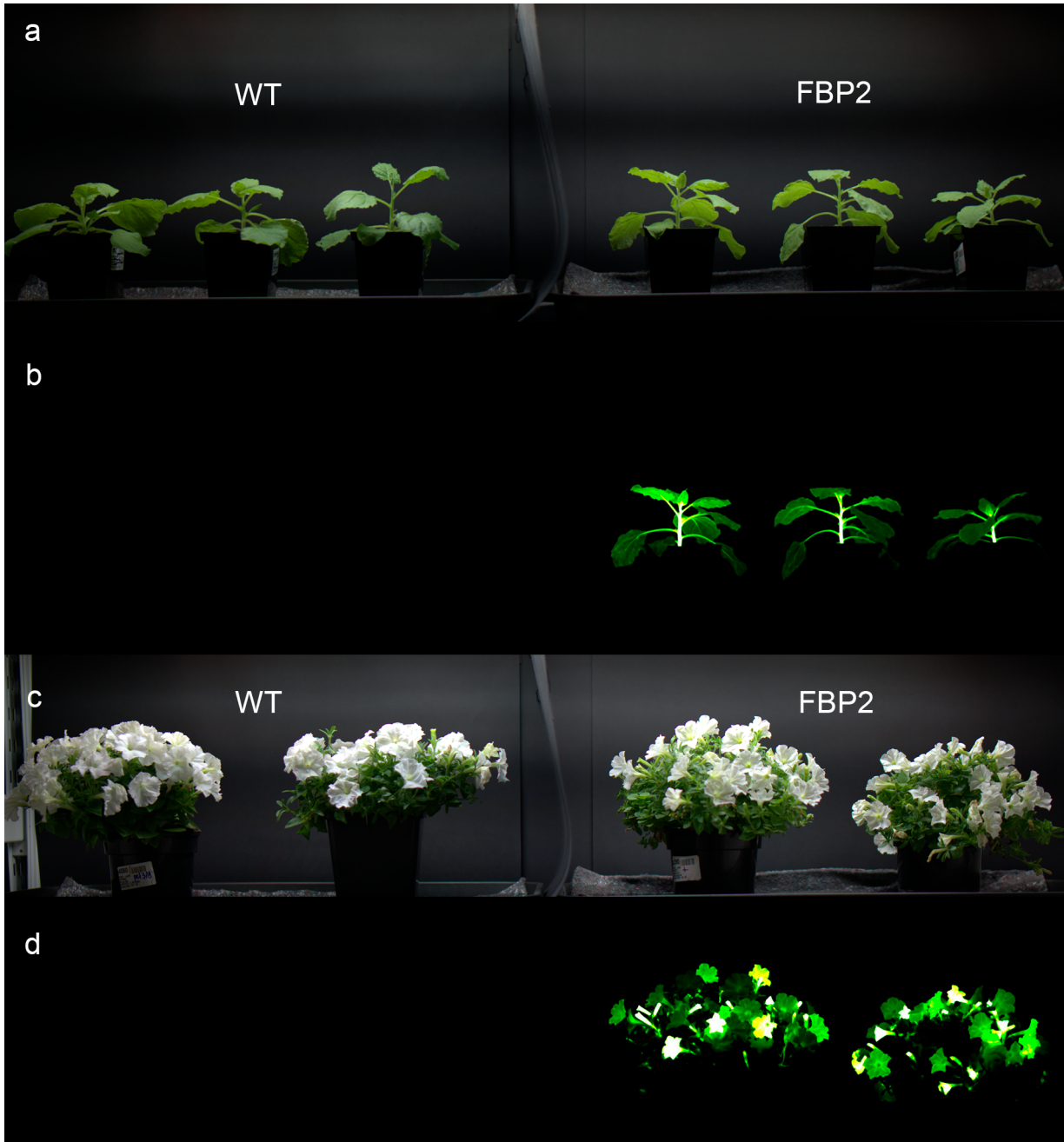
Supplementary Figure 20. Average luminescence of leaves of 6-week-old *A. thaliana* transformed with FBP2 and FBP3 pathways. The photo (ISO 400, exp - 30 sec)(a) and average luminescence of leaves(b). The boxes are the first and the third quartiles, whiskers are the rest of the distribution except outliers, the orange line is the median. The colour of data points indicates different plant lines (2 lines for FBP2, 3 lines for FBP3 per box plot); N = 20 or 40 leaves for FBP2 or FBP3, respectively. The difference between mean values and p-value of post-hoc two-sided Mann-Whitney U-test are indicated below the brackets between the box plots, $p = 1.8e-09$.



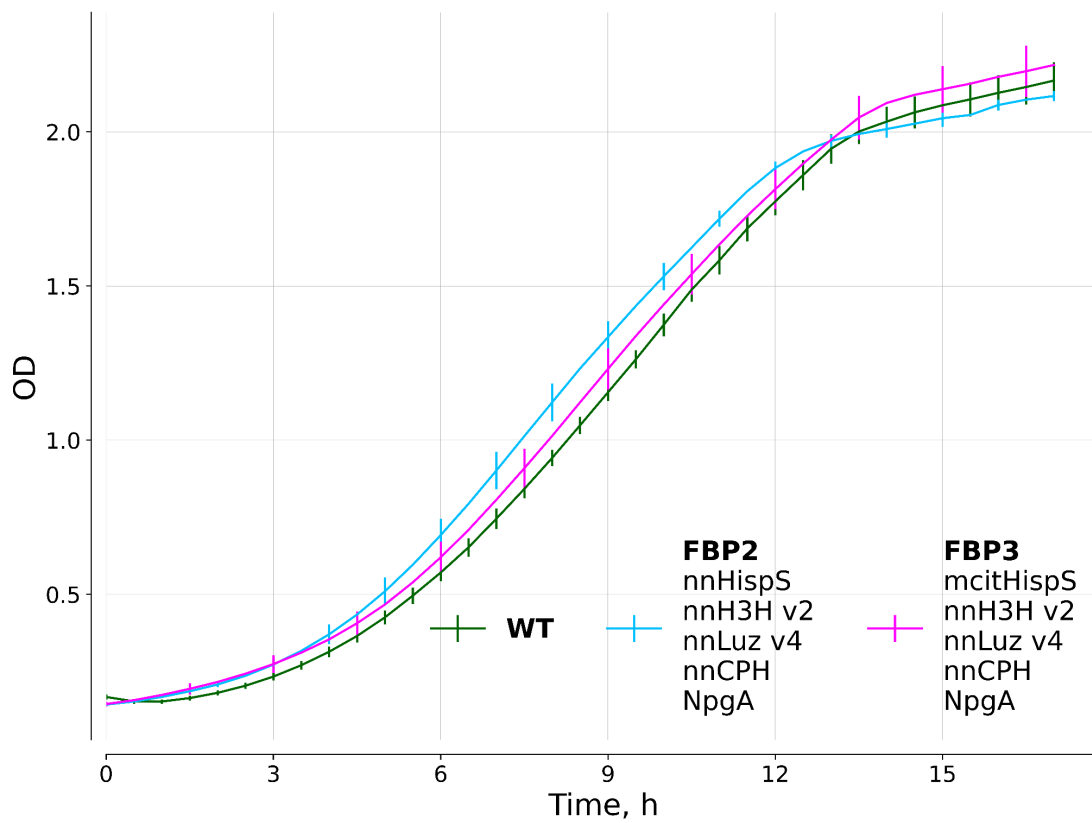
Supplementary Figure 21. Imaging of *P. hybrida* transformed with FBP2 and FBP3 pathways. ISO 3200, exposure – 30 sec.



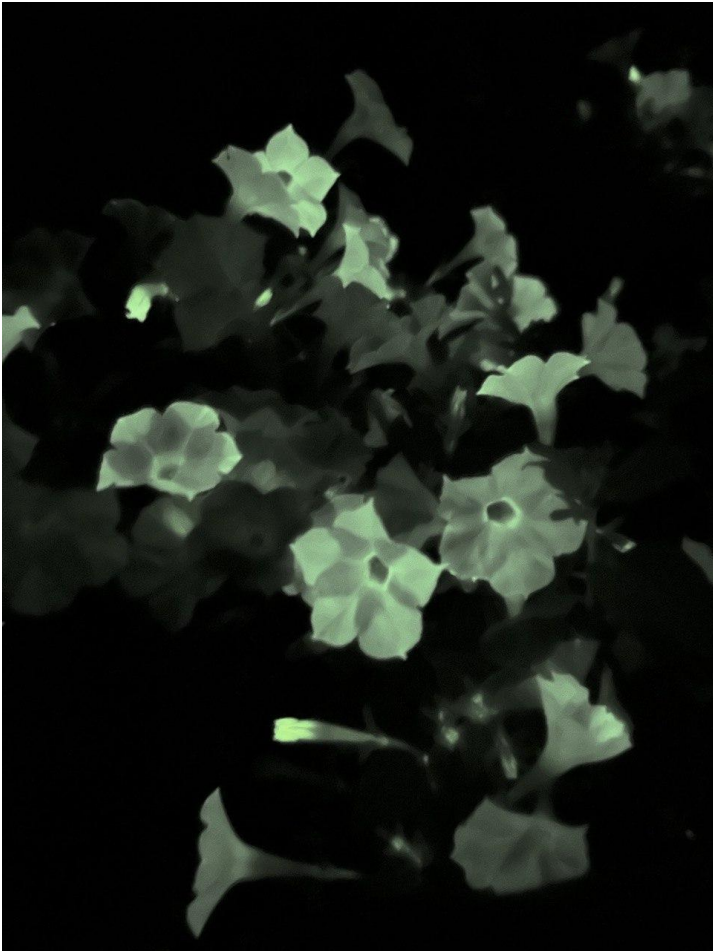
Supplementary Figure 22. Imaging of flowers of *P. hybrida* transformed with FBP2 pathway (out of the black paper box), in the black paper box - control flowers of *P. hybrida* WT, values next to ROI represent average radiance (p/s/cm²/sr).



Supplementary Data Figure 23. Phenotype of glowing and wild-type *Nicotiana benthamiana* and *Petunia hybrida* plants. Potted wild-type and glowing plants of the same age in ambient light (a, c) and in the dark (b, d).



Supplementary Figure 24. The growth of the yeast strain *P. pastoris* GS115 WT and strains expressing FBP1 or FBP3 pathways. N = 1 yeast strain for WT, 1 for FBP2 and 2 yeast strains for FBP3. N = 3, 6 and 12 biologically independent replicates for WT, FBP2 and FBP3, respectively. Data shown as mean (solid line) \pm SD (whiskers).



Supplementary Figure 25. A non-edited photo of FBP3-expressing T3 petunia line, shot on iPhone 14 Pro.



Supplementary Figure 26. A non-edited photo of FBP3-expressing yeast strain in media supplemented with caffeic-acid, shot on iPhone 14.

Supplementary Video captions

Supplementary Video 1. A non-edited video of FBP3-expressing yeast strain in media supplemented with caffeic-acid, shot on iPhone 14. 26 frames per second, 1920x1080 px.

Supplementary Video 2. Video-rate luminescence imaging of transgenic plants expressing FBP2.

Supplementary Video 3. A non-edited video of FBP3-expressing T3 petunia line, shot on iPhone 13 Pro. 27 frames per second, 1920x1080 px.

Supplementary Table 1. Plasmids used in this study.

Gene	Plants	HEK293NT	<i>P. pastoris</i>
NpgA	pX131 https://benchling.com/s/seq-j7hk6Mo26QwL2oWED3un?m=slm-HdHkCgJfryAnOv6asct4		
CPH	pX020 https://benchling.com/s/seq-6LZLUoXSSOLiGcl.sf4Kh?m=slm-FYDYDjEJRJS3k5i8xCim Plasmid from the Ref ¹		
nnHispS	bent pX021 https://benchling.com/s/seq-dgVnzNzzdb1O8UOlwsyO?m=slm-idFOm0ekAOKLIE1D6Wn8 pX022 https://benchling.com/s/seq-HfeqUXOckkj1jMpb8yjl?m=slm-KGFqWOofY1Og47FNvgoW Plasmids from the Ref ¹	hum https://benchling.com/s/seq-byUf6AubNNzHTRvJWeNE?m=slm-ZGRq9MG6HGKrTHLR5MOP	ppas pNK4498 https://benchling.com/s/seq-0gFj0FeLuSEPOTReGrGi?m=slm-zpt87PSoadoe2DyTihdO
afHispS from <i>Armillaria fuscipes</i>	ppas/bent/hum pNK6009 https://benchling.com/s/seq-HpX0quRopNzuXCbkF5Og?m=slm-orj900TrXMnI4gONdXJF		
agHispS from <i>Armillaria gallica</i>	ppas/bent/hum pNK2655 https://benchling.com/s/seq-B3s5evCuyPshI06CKV74?m=slm-LfzIK93bmq7cDAJLcgSd		
amHispS from <i>Armillaria mellea</i>	ppas/bent/hum pNK2656 https://benchling.com/s/seq-LDE3DqzjVztM8DqiQ0P?m=slm-9TE0Rs2JHhX2nvAUdFrT		
aoHispS from <i>Armillaria ostoyae</i>	ppas/bent/hum pNK2652 https://benchling.com/s/seq-7nLForLwJbaAC979vsFp?m=slm-UCKyNedQWh16FeOoNYeZ		
gnHispS from <i>Guyanagaster necrorhiza</i>	ppas/bent/hum pNK2719 https://benchling.com/s/seq-StnFUnRATVrtB8pA86S2?m=slm-iy2iuDGpOiMtNCcT25uU		
mchHispS from <i>Mycena chlorophos</i>	ppas/bent/hum pNK2653 https://benchling.com/s/seq-6CYJYzhIFKzsRPW7Rz9V?m=slm-FJDKAWwsRYmNn1htk0BL		
mcitHispS from <i>Mycena citricolor</i>	ppas/bent/hum pNK2657 https://benchling.com/s/seq-xd4LQ0hTXWIL5RMO7yqa?m=slm-HDLsqpEWOOXnf12rOno5		
ngHispS from <i>Neonothopanus gardneri</i>	ppas/bent/hum pNK2842 https://benchling.com/s/seq-1M2h5oTlyutLITu1F2ai?m=slm-VA5fzuD7R0z9l6mJjqlf		
psHispS #1 from <i>Panellus stipticus</i>	ppas/bent/hum pNK2659 https://benchling.com/s/seq-VCK6vPdlaky1ZplEvAFI?m=slm-7rKLyhfDI3xwQysqyZ0N		

psHisP #2 from <i>Panellus stipticus</i>	ppas/bent/hum pNK2660 https://benchling.com/s/seq-1VmaBYvYwc93bfHoWYN8?m=slm-1N6afQYNHnz3xnA2hD8j		
nnH3H WT	bent pX019 https://benchling.com/s/seq-ZVjoLQvL9ipOb0Jdx0PW?m=slm-UEsBpjNhXxwZpv9tta4E Plasmid from the Ref ¹	hum pNK180 https://benchling.com/s/seq-ZGuQ24Ks9ONH5OuPDrmR?m=slm-h01x8hemK2Co5EiA501V	ppas pNK4404 https://benchling.com/s/seq-nl9IN0MCpySO3k0Thj3P?m=slm-s5Y3vHxsDwaH7jYge0gv
nnH3H v2	bent pNK116 https://benchling.com/s/seq-TVjgbAUDpSLtXwiAPO3g?m=slm-ExzXht21ELkHozzykx8B	hum pNK189 https://benchling.com/s/seq-L4r5kVGHb1f7hBXDCThE?m=slm-pMFOFMzgm6ge3876qxah	ppas pNK4169 https://benchling.com/s/seq-aRCkab6wDjYyXaRkdFYw?m=slm-zlyvnieVKI8rR9MrHo3v
nnLuz WT	bent pX018 https://benchling.com/s/seq-OJbc805At80x2L7dguDN?m=slm-FxbJkfNO6T8ibDTovi4U Plasmid from the Ref ¹	ppas/hum pNK5756 https://benchling.com/s/seq-VtvFVR5UJ7K1KfrVM2qb?m=slm-5hjDOc0iqHqSRDiPzWJl	
nnLuz v4	bent pNK115 https://benchling.com/s/seq-y3dUN4bGeogUKptVuQyK?m=slm-KBkqCwCQ64I3uPpx8C6a	ppas/hum pNK845 https://benchling.com/s/seq-aMJVqAQHeop5RyZR3hia?m=slm-ew9YhTknPhMrPNEUICof	

Supplementary Table 2. Plasmids for expression in mammalian cells.

ID	Plasmid description	Plasmid map
pNK5780	DVK_AF pCMV - nnLuz WT ppas/hum - tSV40	https://benchling.com/s/seq-9tRkFeQVsuOn2Py3DrF4?m=slm-8KKhqejiF99jNeFc0Mx
pNK5664	DVK_AF pCMV - nnLuz v4 ppas/hum - tSV40	https://benchling.com/s/seq-szpjIEFox2RoBAzbCX12?m=slm-ihCzRTcKpFU1ZiX3PXHU
pNK294	DVK_AF pCMV - nnH3H WT hum - tSV40	https://benchling.com/s/seq-DzJM6LiezPSHLR66y00a?m=slm-nifL6L9bWmLeokWZxID
pNK291	DVK_AF pCMV - nnH3H v2 hum - tSV40	https://benchling.com/s/seq-XBaFaZS0wkvLAYwv2xb?m=slm-YO3g3Fqr27hzRjO305Jl
pX136 Plasmid from the Ref ¹	C-vector pCMV - CPH - tSV40	https://benchling.com/s/seq-TsINsVoYeWAlr0I8LE2B?m=slm-IK2nwHwYI62YHGfn66OL
pX137 Plasmid from the Ref ¹	C-vector pCMV - npgA - tSV40	https://benchling.com/s/seq-scdZF7tmlp3uwCvN5cq5?m=slm-Kks6Aspwz3PogvacsPy
pNK6006	DVK_AF pCMV - nnHisps hum - tSV40	https://benchling.com/s/seq-VFZ8LR01nGvooM2ZpFnU?m=slm-i5txnmCnrwnLcituwEQ
pNK3203	DVK_AF pCMV - mcithisps - tSV40	https://benchling.com/s/seq-Sl4CaS08bfKDONntgU7w?m=slm-2CiaPJMFeqOFmn17jpEK
pNK3205	DVK_AF pCMV - aoHisps - tSV40	https://benchling.com/s/seq-GrSF5SUBkagxp2llhnPQ?m=slm-6LBWbRoQOW6JxSoFb60Q
pNK3209	DVK_AF pCMV - gnHisps - tSV40	https://benchling.com/s/seq-4x5o1iTEz6fzVanqlOPp?m=slm-RW4RC1IOF1cCNI620uWn
pNK3210	DVK_AF pCMV - agHisps - tSV40	https://benchling.com/s/seq-iVe4IVD0pIINdsP9vgHD?m=slm-OrPPzVKHs4hh8xOpz3IV
pNK3061	DVK_AF pCMV - amHisps - tSV40	https://benchling.com/s/seq-Thpig3qd8mE6wqF8S9IH?m=slm-7d4kin7cQpAcxWxnZJKf
pNK6009	DVK_AF pCMV - afHisps - tSV40	https://benchling.com/s/seq-46AvNq6LGOdOUIDBUN3w?m=slm-UXc1INm2SzBdDm7kOkqR
pNK6265	DVK_AF pCMV - FFLuc - tSV40	https://benchling.com/s/seq-7Xlv3YGxuGpSS2LwOtL1?m=slm-BAthmBp9g0mgHBNRXE9U
pNK6272	DVK_AF pCMV - nanoLuc - tSV40	https://benchling.com/s/seq-hKlxkDzoUtyCV2BNSFGG?m=slm-ak5BeGANecNrrh2YBCMy
pNK6593	pCIDAR pCMV - co-iLuxA - tSV40	https://benchling.com/s/seq-gv6bAgIxSUKo7oL6fSpC?m=slm-Qzm79rNdEhJXqfIS0wdA
pNK6595	pCIDAR pCMV - co-iLuxB - tSV40	https://benchling.com/s/seq-HMiGiWoHkFY8FBW98euO?m=slm-2FSrSwfQto2PRzHxLjlf
pNK6599	pCIDAR pCMV - co-iLuxC - tSV40	https://benchling.com/s/seq-Qpxe6tYz0yodSVOdQxV2?m=slm-OKpckjebRkuwvjv09WMMK
pNK6612	pCIDAR pCMV - co-iLuxD - tSV40	https://benchling.com/s/seq-igTww6g9sStn6rNCfPIh?m=slm-eEGfZCyUnLiqxgWe89l
pNK6604	pCIDAR pCMV - co-iLuxE - tSV40	https://benchling.com/s/seq-CLqFGnVM6M6SmYjN3UUG?m=slm-JSqj8xAqU8BNcmVf3S1j

pNK6614	pCIDAR pCMV - frp - tSV40	https://benchling.com/s/seg-R23icci4Bl8jYpD6GdTX?m=slm-FMb8Ll5vgoUsUxeBil2t
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Supplementary Table 3. Plasmids for expression in *P. pastoris*.

ID	Plasmid description	Plasmid map
pNK1508	pGAP-Hyg NpgA	https://benchling.com/s/seq-wSZV0ADJQVfKbmbkxI0Y?m=slm-sq6RcovW5i00rsL5RbWt
pNK5869	pGAP-Z nnHispS	https://benchling.com/s/seq-8YnNqUjSobFUjkreIKNu?m=slm-RUJJJdkJIV0g1aTaV5D9m
pNK3293	pGAP-Z afHispS	https://benchling.com/s/seq-4ifheFWnNITRMyl1PwWTP?m=slm-dyPZ8ct3kU3f2Fezqz4C
pNK5871	pGAP-Z agHispS	https://benchling.com/s/seq-08n0A9ZKqB7yHbllNZef?m=slm-oz7B27p56mnio4h6l3op
pNK5889	pGAP-Z amHispS	https://benchling.com/s/seq-sfMSGdiOivMc2ryRTMaw?m=slm-czE8bLlmiHfq03xIGN1j
pNK5913	pGAP-Z aoHispS	https://benchling.com/s/seq-thvcdYtpRkUrBoZue81q?m=slm-TBlqYJmPQSVJONDxGVuU
pNK3292	pGAP-Z gnHispS	https://benchling.com/s/seq-u8RO6pOdToKTUezy0tNp?m=slm-rzF6lQODcZhEocnRlfn
pNK3287	pGAP-Z mchHispS	https://benchling.com/s/seq-2Rum32em3qllcnp2fHOA?m=slm-vN5VBNmZLjkLobrLTrIE
pNK5867	pGAP-Z mcitHispS	https://benchling.com/s/seq-lVcfX0nBxssWmeqXvt4p?m=slm-xcpaq3j8GRPGql_4HeaPq
pNK3222	pGAP-Z ngHispS	https://benchling.com/s/seq-MTitklOHQIMVTPaDI0J2?m=slm-jhVXTYbOYKaiAulldHK4
pNK3017	pGAP-Z psHispS #1	https://benchling.com/s/seq-TXzLZk0INjCNEIKCzDFw?m=slm-appoL_NampXAJ4zTUW0Xn
pNK3019	pGAP-Z psHispS #2	https://benchling.com/s/seq-F4vpXaWNjX1JIVko5gf7?m=slm-OpJMfSwnxB4zgdPGuB66
pNK5709	pGAP-Hyg nnH3H WT	https://benchling.com/s/seq-01lxHpymMnUomzl3TZ4E?m=slm-tn4sNABx3A5f0dh01DFq
pNK5712	pGAP-Hyg nnH3H v2	https://benchling.com/s/seq-7tWRDMIL0skZFAMTJpEW?m=slm-1R8J6U1iC3NIMvznk5lu
pNK5788	pGAP-Kan nnLuz WT	https://benchling.com/s/seq-7CxEVTT20bRKNS3m6yM8?m=slm-61XdsunkiFEZRFMTNEo
pNK5785	pGAP-Kan nnLuz v4	https://benchling.com/s/seq-UuMufCjCBXGciWrr2IG2?m=slm-OW3bR80rxCmEdPJaoJr9
pNK6624	pGAP-Hyg nnLuz WT - HiBiT	https://benchling.com/s/seq-Amwp8L7rUC8h5RuCcu6V?m=slm-l8TtpZ0t5p5S.JN1Zpxbf
pNK996	pGAP-Hyg nnLuz v4 - HiBiT	https://benchling.com/s/seq-HXE8ZVTGkGFG8I0ci6Uc?m=slm-8lTOVDp1czXYE4v8tg7N

Supplementary Table 4. Plasmids for expression in plants.

ID	Plasmid description	Plasmid map
pN094	Level 1 p35s - NpgA - tOCS	https://benchling.com/s/seq-o1FM7PTx7f1izd0G5Zte?m=slm-Jtuehn5Ny7J7hoaUjYQZ
pNK077	Level 1 pCmYLCV - NpgA - tATP	https://benchling.com/s/seq-Pru2MS278Nrr7yxjNQBK?m=slm-txwiWQz5LC3quw08eTel
pX028	Level 1 p35s - nnHisps - tOCS	https://benchling.com/s/seq-PigSpOXySuN3KAXDS4dp?m=slm-4KWYBc2Yib5mOyflX6z6
pNK2714	Level 1 p35s - mcitHisps - tOCS	https://benchling.com/s/seq-OTdGulhG7uBSySd06gMy?m=slm-5GhfWLPdsw4YcJ5tad2D
pNK2748	Level 1 p35s - gnHisps - tOCS	https://benchling.com/s/seq-NxvVsys9zCAFolkKhApg?m=slm-PB4MJ2q1q1oaChmNrnj4
pNK5739	Level 1 p35s - afHisps - tOCS	https://benchling.com/s/seq-co3aAC8InccrH6PLg6ao?m=slm-YjFuVC00C0dBatCFGog8
pNK2674	Level 1 p35s - aoHisps - tOCS	https://benchling.com/s/seq-4QYpwefWZvmOhWBtxGya?m=slm-axGMB89CJrSpn9pO8NP5
pNK2711	Level 1 p35s - agHisps - tOCS	https://benchling.com/s/seq-FHSP8wG3lmlLj1fbh3Sl?m=slm-5wOHQUJA3FHZdkrL8btD
pNK2816	Level 1 p35s - amHisps - tOCS	https://benchling.com/s/seq-6wllrkjhFRYO4wkBfDVU?m=slm-hOWhMPCl500AjtHHjeWV
pNK2715	Level 1 p35s - psHisps #1 - tOCS	https://benchling.com/s/seq-8GEGPbckUqblDANTIVhQ?m=slm-vzz7g32kx8Nbs5xngO6
pNK2716	Level 1 p35s - psHisps #2 - tOCS	https://benchling.com/s/seq-KzI5qzOPg8jkd8edxE7J?m=slm-hYP8T6y9ml8SHIDBE2gC
pNK2857	Level 1 p35s - ngHisps - tOCS	https://benchling.com/s/seq-Ca00IIPJ46K8FZ0l0Fwd?m=slm-HhAV8lhpaKtZiFjiE02
pNK2676	Level 1 p35s - mchlHisps - tOCS	https://benchling.com/s/seq-PEYW7vARjkcANQjioo6?m=slm-W1J3vqyauF5ycDGgBNL
pNK093	Level 1 pFMV - nnH3H WT - tNOS	https://benchling.com/s/seq-DnBkfiWoak29sqieFLof?m=slm-U5OjKtgT09Z9dMdlClrw
pNK156	Level 1 pFMV - nnH3H v2 - tNOS	https://benchling.com/s/seq-j5jkkjuRK5VNLsbXtGp?m=slm-aFvsHms19B6JefbsmpoG
pNK5668	Level 1 p35s - nnLuz WT - tAct2	https://benchling.com/s/seq-ikgdQelPsEaWW9MUfwc2?m=slm-6lli6zm0m4eWaNoON453
pNK155	Level 1 p35s - nnLuz v4 - tAct2	https://benchling.com/s/seq-JHhqKcFYX05LiRKLlTaa?m=slm-YYutXmSCiqovJHglSB1
pX030	Level 1 p35s - nnCPH - tOCS	https://benchling.com/s/seq-7XBDGX8yci30y36lnhGw?m=slm-76s6l2kobMN6e46mKQkd
pNK6260	Level 1 p35s(0.4 kb) - FFLuc - tOCS	https://benchling.com/s/seq-rLXcKcvdkt8DpM9o7Xcq?m=slm-oEP8R0c7iWC65YScfAIF
pNK6269	Level 1 p35s(0.4 kb) - nanoLuc - tOCS	https://benchling.com/s/seq-BKRLDkmTAUWRNmfBmHUj?m=slm-SCGfQZMUgicllOxp4sC4
pNK6357	Level 1 p35s(0.4 kb) - iLuxA - tOCS	https://benchling.com/s/seq-5Whqyp9xxIKYwcy7IK4X?m=slm-Mqowq7sYFAWAZauGiMon

pNK6359	Level 1 p35s(0.4 kb) - iLuxB - tOCS	https://benchling.com/s/seq-iGulTHkamFwT15pJwMr6?m=slm-uL9SwDh90Cl2zzK95cdS
pNK6361	Level 1 p35s(0.4 kb) - iLuxC - tOCS	https://benchling.com/s/seq-HhJczSCOXbtrDqscfakv?m=slm-354btwib8r1hMXylnzVj
pNK6363	Level 1 p35s(0.4 kb) - iLuxD - tOCS	https://benchling.com/s/seq-X5tMVTn10Ll0yHXxXnzX?m=slm-TLciHUgakLS3DGq0FdLL
pNK6365	Level 1 p35s(0.4 kb) - iLuxE - tOCS	https://benchling.com/s/seq-04VdDNUXuThYsrm9J5zk?m=slm-nSOsz93zbbVpFWFwy9Ql
pNK6367	Level 1 p35s(0.4 kb) - frp - tOCS	https://benchling.com/s/seq-Zc7BGRR0psslBkAvocrn?m=slm-pRsZjLtm8waki3ukLPjo
pNK6369	Level 1 p35s(0.4 kb) -chloroplast_transit_peptide-iLuxA - tOCS	https://benchling.com/s/seq-xKBn7U0j4YrLkSVk0eYK?m=slm-bVS3mBcnBo03Vuzln1IF
pNK6371	Level 1 p35s(0.4 kb) -chloroplast_transit_peptide-iLuxB - tOCS	https://benchling.com/s/seq-rZYCXpgDbgnwUQRAuxl2?m=slm-l5857JDdPVz95vCGJM1o
pNK6373	Level 1 p35s(0.4 kb) -chloroplast_transit_peptide-iLuxC - tOCS	https://benchling.com/s/seq-rwCYI60SeCBI5kMeSfkq?m=slm-fncQqex2ookrBWzOpVl8
pNK6375	Level 1 p35s(0.4 kb) -chloroplast_transit_peptide-iLuxD - tOCS	https://benchling.com/s/seq-oDmtu6doZzDV1U1asoRB?m=slm-lyDksiP3zGwNiluVap2j
pNK6377	Level 1 p35s(0.4 kb) -chloroplast_transit_peptide-iLuxE - tOCS	https://benchling.com/s/seq-u1D4fbNnAqPQrIBKY1w6?m=slm-Xh3JTMwHXJcfEg55R1nb
pNK6379	Level 1 p35s(0.4 kb) --chloroplast_transit_peptide-frp - tOCS	https://benchling.com/s/seq-U65pjinwhXL2EjjZf311L?m=slm-WC7YkOy3Sij4YSH7ox7u
pNK511	nnHispS, nnH3H_v2, nnLuz_v4, nnCPH Level P pNOS - KanR - tOCS p35s - nnHispS - tOCS p35s - Luz v4 - tAct2 p35s - nnCPH - tOCS pFMV - nnH3H v2 - tNOS	https://benchling.com/s/seq-GTulg6Rx7L3ISXacqilq?m=slm-h322lbfm0Y36Q9rbSwyA
pNK497	FBP2 Level P pNOS - KanR - tOCS p35s - nnHispS - tOCS pCmYLCV - NpgA - tATP p35s - Luz v4 - tAct2 p35s - nnCPH - tOCS pFMV - nnH3H v2 - tNOS	https://benchling.com/s/seq-DgozAu4sYn937p5UdD6Z?m=slm-m38crKINPSj2Sg8dkAjq
pNK3071	FBP3 Level P pNOS - KanR - tOCS p35s - mcitHispS - tOCS pCmYLCV - NpgA - tATP p35s - Luz v4 - tAct2 p35s - nnCPH - tOCS pFMV - nnH3H v2 - tNOS	https://benchling.com/s/seq-NNKqOeRGCYTISxRYy3SQ?m=slm-78WwJlaPG6mU0O6gRBCC
pNK6537	mcitHispS, nnH3H_v2, nnLuz_v4, nnCPH, NpgA Level P pNos - KanR - tOCS p35s - mcitHispS - tOCS pFMV - nnH3H_v2 - tNOS p35s - nnLuz_v4 - tAct2 p35s - nnCPH - ocsT pCmYLCV - npgA - tATP	https://benchling.com/s/seq-ihOTINEWjRUG85MDv5Ng?m=slm-npKsDdUw7TzPk38MbvYV
pNK6541	nnHispS, nnH3H_v2, nnLuz_v4, nnCPH, NpgA Level P pNos - KanR - tOCS p35s - nnHispS - tOCS pFMV - nnH3H_v2 - tNOS p35s - nnLuz_v4 - tAct2 p35s - nnCPH - ocsT pCmYLCV - npgA - tATP	https://benchling.com/s/seq-l5IRAnDAKaZtcokLeGbe?m=slm-OJwidfOKcolTkzZFRuqJ

Supplementary Table 5. Transgenic plant lines used in this study.

Plant species	Plasmid, pathway	Line ID	
<i>Arabidopsis thaliana</i>	pNK497, FBP2	AT6121	
		AT6123	
	pNK3071, FBP3	AT6124	
		AT6125	
		AT6126	
	<i>Chrysanthemum morifolium</i>	pNK497, FBP2	CHR2105
<i>Nicotiana benthamiana</i>	pX037, FBP1	NB021 from ref ¹	
	pNK511	NB097	
		NB253	
		NB1291	
	pNK497, FBP2	NB072	
		NB081	
		NB103	
		NB104	
	pNK3071, FBP3	NB2186	
		NB5730	
		NB5732	
		NB5733	
		NB5734	
	<i>Nicotiana tabacum</i>	pX037, FBP1	NT001 from ref ¹
		pNK497, FBP2	NT2641
			NT2817
NT2819			
<i>Petunia hybrida</i>	pNK497, FBP2	PET373	
	pNK3071, FBP3	PET2720	
		PET2234	
<i>Populus canadensis</i>	pX037, FBP1	POP5	
	pNK497, FBP2	POP11	

Supplementary information references

1. Mitiouchkina, T. *et al.* Plants with genetically encoded autoluminescence. *Nat. Biotechnol.* (2020) doi:10.1038/s41587-020-0500-9.