SUPPLEMENTARY MATERIAL

METHODS

sgRNA design. The complementary DNA sequence of the direct repeat that is recognized by dCas13a from *L. wadei* is GATTTAGACTACCCCAAAAACGAAGGGGACTAAAAC [1]. The corresponding spacer is 30 nucleotides long and is located in the 3' end. A new direct repeat after the spacer and before the transcription terminator provokes the maturation of the sgRNA by dCas13a (as the wild-type protein has two distinct RNase activities) [2] to generate clean spacers. Different genetic architectures for sgRNA expression were constructed and assayed (without additional direct repeat and with a ribozyme after the spacer). The spacers of the sgRNAs were simply taken as the reverse complement of the targeted sequences. We designed sgRNAs to target the *sfGFP* and *eBFP2* genes. In the case of activation of translation initiation, the spacer was designed to induce a conformational change in the 5' UTR of the target mRNA [3]. Here, the transcription terminator from the *E. coli* gene *rrnC* was employed, as done elsewhere [4].

Genetic constructions. The complete DNA synthesis of *dCas13a* from *L. wadei* (having the mutations R474A and R1046A, which disable the RNA-guided single-stranded RNA cleavage activity) [1] was done by GenScript (codon-optimized for *E. coli*), then cloned under the control of the T5-*lac* promoter into a plasmid with origin of replication pBR322 and resistance to ampicillin (pQE-60). The different sgRNAs were synthesized as linear DNA fragments by IDT or Sigma, subsequently cloned into a plasmid with origin of replication pSC101(E93G) and resistance to kanamycin [4]. However, for *in vivo* experiments, the sgRNAs were cloned into the plasmid harboring the *dCas13a* digesting with NheI. The *sfGFPs* with different 5' UTRs were synthesized as linear DNA fragments by IDT, which were then cloned into a plasmid with origin of replication pUC and

resistance to ampicillin (pUCIDT) for *in vitro* experiments and into a plasmid with origin of replication p15A and resistance to chloramphenicol (p15Cat) for *in vivo* experiments [5]. The *eBFP2s* with different 5' UTRs were synthesized as plasmids by IDT with origin of replication pUC and resistance to ampicillin (pUCIDT). All sgRNAs, sfGFPs, and eBFP2s were expressed from a constitutive promoter (J23119). In addition, *E. coli* Dh5α was used as a strain for cloning purposes. Plasmids were amplified through overnight bacterial cultures in LB at 37 ºC and 220 rpm (Dh5α for the plasmids encoding sgRNAs or sfGFPs and MG1655-Z1 for the plasmid encoding dCas13a). Ampicillin was used at 100 µg/mL, kanamycin at 50 µg/mL, and chloramphenicol at 34 µg/mL. Plasmids were then retrieved by miniprep (Thermo), followed by purification with a PureLink kit (Thermo). Finally, they were quantified in a Nanodrop (Thermo).

RNA folding. The cofold routine from the ViennaRNA webserver [6] was used to predict the ΔG value for the different sgRNA-mRNA interactions. This was run with default parameters, except by setting the temperature to 29 ºC. To get the ΔG distributions for each mechanism of translation control, random 5' UTRs were generated with the inverse folding routine with sequence and structural constraints.

Cell-free expression. *In vitro* transcription-translation was run with the myTXTL protein expression kit (Arbor), based on an *E. coli* cell extract, amino acids, and an energy buffer [7]. Low DNA binding 1.5 mL tubes (Sarstedt) were used as reaction containers, with 9 µL of σ70 master mix and an appropriate volume of each plasmid, then adjusting with nuclease-free water to 12 µL. To assess the repression or activation of sfGFP, the plasmid encoding the sfGFP was placed at 2 nM, the plasmid encoding the dCas13a at 3 nM, and the plasmid encoding the sgRNA at 6 nM. To assess the orthogonal regulation of eBFP2, the plasmid encoding the eBFP2 or sfGFP was placed at 2 nM in the case of repression

and 3 nM in the case of activation, the plasmid encoding the sgRNA at 10 nM in the case of repression and 6 nM in the case of activation, and the plasmid encoding the dCas13a always at 3 nM. These concentrations may need adjustment depending on the expression kit. The tubes were incubated for 16 h at 29 ºC in a stove (Incudigit; Selecta). Reactions were stopped by placing the tubes on ice. Some marginal evaporation was observed, but no condensation on the lid. Reactions were done in triplicate.

Fluorescence quantification. Incubated samples of cell-free expression (about 12 µL) were transferred to a microplate (384 wells, black, clear bottom; Falcon) for characterization. The microplate was assayed in a fluorometer (Varioskan Lux; Thermo) to measure green fluorescence (excitation at 485 nm, emission at 535 nm). The mean background value of fluorescence corresponding to the cell extract expressing only dCas13a was subtracted to correct the signals and get an estimate of protein expression.

Molecular beacons. Two different RNA beacons (folding into a stem-loop structure) were designed [8], one to prove the interaction associated with the repression of initiation and another the activation of initiation. A fluorescent dye (fluorescein) was placed in the 5' end and a dark quencher (Iowa black) in the 3' end. To reach the appropriate folding, RNA beacons were first heated for 2 min at 95 ºC and then slowly cooled to 25 ºC. On incubated samples of cell-free expression (of dCas13a and sgRNA only), 250 nM of RNA beacon was added. Samples were incubated for additional 5 min at 29 ºC and then quantified.

Bacterial colony assays. LB-agar plates seeded with *E. coli* MG1655-Z1 cells co-transformed with suitable plasmids (a plasmid harboring the *dCas13a* and sgRNA and another plasmid harboring the *sfGFP*) were grown overnight at 37 ºC. Glucose (0.4%) and

lactose (1 mM) were used as supplements. The plates were irradiated with blue light and images were acquired with a 2.8 Mpixel camera with a filter for green fluorescence in a light microscope (Leica MSV269). The commercial software provided by Leica was used to adjust the visualization of the differential fluorescence among plates. The fluorescence intensity of the colonies was quantified with Fiji [9] with appropriate background subtraction.

TECHNICAL NOTES

Note S1. It would be reasonable to accept that the expression of dCas13a would have some nonspecific, but negative effect on sfGFP expression as a result of the competition for myTXTL resources. Our results are not conclusive in this matter, as we also noticed variability in the fluorescence measurements, especially when the plasmid encoding dCas13a was not added to the reaction.

Note S2. We explored the *in vivo* performance of the designer systems with liquid cultures in exponential phase (using minimal M9 medium), but we noted growth defects and marginal regulation, especially in the case of repression. The expected high activity of the ribosomes in exponential phase and the potential existence of unforeseen binding regions in the host transcriptome might explain this behavior. Further work should be conducted to study which genetic background (type of strain) and environmental conditions (carbon source, temperature, *etc.*) are more favorable for the CRISPR-Cas13 system in *E. coli* or even other bacterial cells.

REFERENCES

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SEQUENCES

We provide the nucleotide sequences of the different elements that we used in this work.

> Coding region for dCas13a (from *L. wadei*, codon-optimized for *E. coli*):

```
ATGAAAGTAACTAAAGTCGATGGTATCAGTCATAAAAAGTATATCGAGGAAGGAAAGTTGGTGAAGTCAACAAGTGAAGA
GAATCGCACGTCGGAACGTTTATCCGAATTATTAAGTATCCGCTTAGACATTTACATCAAGAATCCGGATAACGCATCAG
AAGAGGAAAATCGTATCCGCCGTGAGAACTTGAAAAAATTCTTTAGTAACAAAGTACTGCATTTAAAGGACTCCGTGCTT
TACCTGAAGAATCGCAAGGAGAAAAATGCAGTCCAGGATAAAAACTACTCAGAAGAAGACATCAGTGAATACGACTTAAA
GAACAAGAACTCGTTCTCCGTGTTGAAGAAGATTTTACTTAACGAAGACGTAAATAGCGAAGAGCTGGAGATTTTCCGTA
AAGACGTGGAGGCGAAGCTGAATAAGATCAACAGTCTTAAGTATTCCTTTGAGGAGAATAAAGCCAACTATCAGAAAATC
AATGAAAACAATGTAGAAAAGGTTGGCGGAAAGTCTAAGCGCAATATCATCTATGATTACTATCGCGAGTCTGCGAAACG
CAATGATTACATCAACAATGTACAGGAGGCCTTCGACAAACTGTATAAGAAAGAGGATATTGAAAAATTGTTCTTCCTGA
TCGAAAATTCAAAGAAGCATGAAAAATATAAGATCCGCGAGTACTATCACAAAATTATCGGCCGTAAAAACGATAAAGAG
AATTTCGCGAAGATCATTTATGAAGAAATTCAAAATGTAAACAATATCAAGGAACTTATTGAAAAGATCCCAGATATGTC
GGAACTTAAAAAAAGCCAGGTATTCTATAAATACTACTTAGATAAGGAAGAATTAAATGATAAGAACATCAAATACGCCT
TTTGTCATTTCGTCGAAATTGAGATGTCGCAGCTTTTAAAGAATTACGTCTACAAACGTTTATCTAATATCTCCAACGAC
AAAATCAAGCGCATCTTCGAGTATCAGAACTTAAAGAAATTAATCGAAAACAAGCTGCTGAATAAACTGGATACCTATGT
TCGCAACTGCGGTAAATATAATTATTATCTGCAGGTCGGTGAGATTGCAACCTCGGATTTCATTGCTCGTAACCGTCAAA
ACGAGGCTTTCTTGCGCAATATTATCGGGGTATCGTCAGTGGCATATTTTAGCCTGCGCAATATTTTGGAGACCGAAAAT
GAAAACGGTATCACCGGTCGCATGCGCGGCAAGACTGTAAAGAACAACAAAGGGGAGGAGAAGTATGTGTCAGGAGAAGT
AGATAAGATTTATAATGAAAACAAACAGAACGAAGTAAAGGAAAACTTGAAAATGTTTTATTCGTACGATTTTAATATGG
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AATTTAGAATTGGAGGGTAAAGATATTTTTGCGTTTAAAAATATTGCGCCCAGTGAAATCAGCAAGAAGATGTTCCAGAA
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ATGTGATTATCAAGTACTTGAAGAACACTAAATTCAACTTTGTCAATAAAAATATTCCTTTTGTACCCTCATTCACAAAA
TTATACAACAAGATTGAAGATCTGCGTAACACGCTTAAGTTCTTTTGGTCAGTGCCGAAGGATAAAGAGGAGAAAGACGC
ACAAATTTATTTGCTGAAGAATATTTACTATGGGGAGTTCCTGAACAAATTCGTAAAGAACTCTAAAGTCTTTTTTAAAA
TTACCAACGAGGTTATCAAAATCAATAAACAGCGCAACCAGAAAACTGGCCATTACAAATACCAGAAGTTCGAAAATATT
GAGAAGACGGTTCCAGTTGAGTATTTAGCAATTATCCAAAGTCGTGAGATGATCAACAATCAAGACAAAGAAGAGAAGAA
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AATCTAATAATAACAATGACAACAACGATATTTTTTCGAAAATCAAAATTAAAAAAGATAACAAAGAGAAATATGACAAA
ATCTTGAAGAACTATGAGAAGCATAATCGTAATAAGGAGATCCCCCACGAAATCAATGAGTTTGTGCGCGAGATTAAATT
GGGAAAGATCCTTAAATATACTGAAAACTTGAATATGTTCTATCTTATTTTAAAGTTGTTAAATCATAAAGAGTTAACAA
ACCTTAAGGGTTCCCTGGAGAAGTATCAGAGTGCTAACAAAGAAGAGACGTTTTCAGACGAATTGGAACTGATCAATTTG
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TCGTTGAAAGAGCTTAAGGAATACAGCAACAAGAAGAACGAGATTGAGAAAAACTACACTATGCAACAAAATCTTCATCG
TAAGTATGCCCGTCCGAAGAAGGATGAAAAATTTAATGACGAAGATTATAAGGAGTACGAGAAAGCGATCGGCAATATTC
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TTAAAAAACTTGAAAAAAAAGAAATTAATGACCGACCGCAACTCCGAAGAATTATGCGAGCTTGTCAAAGTAATGTTCGA
GTATAAAGCACTGGAGTAA
```

> Coding region for sfGFP (repression variant):

```
ATGGGATCCGTGAGCAAGCGTAAAGGCGAAGAGCTGTTCACTGGTGTCGTCCCTATTCTGGTGGAACTGGATGGTGATGT
CAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTA
CTACTGGTAAACTGCCGGTACCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCG
GACCATATGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCCTTTAAGGA
TGACGGCACGTACAAAACGCGTGCGGAAGTGAAATTTGAAGGCGATACCCTGGTAAACCGCATTGAGCTGAAAGGCATTG
ACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTGGAATACAATTTTAACAGCCACAATGTTTACATCACCGCCGAT
AAACAAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGATGGCAGCGTGCAGCTGGCTGATCACTA
CCAGCAAAACACTCCAATCGGTGATGGTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTA
AAGATCCGAACGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAA
```

CTGTACAAATAA

> 5’ UTR of the mRNA coding for sfGFP (repression variant):

ATCCCTAGAGAAAGAGGAGAAATACCCG

> Coding region for sfGFP (activation variant):

ATGCACATAATCACTAGTCGTAAAGGCGAAGAGCTGTTCACTGGTGTCGTCCCTATTCTGGTGGAACTGGATGGTGATGT
CAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTA
CTACTGGTAAACTGCCGGTACCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATCCG
GACCATATGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCCTTTAAGGA
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CCAGCAAAACACTCCAATCGGTGATGGTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTA
AAGATCCGAACGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAA
CTGTACAAATAA

> 5’ UTR of the mRNA coding for sfGFP (activation variant):

ACCATGAAATGCGAGCCGGGAATTGATATTGTGATTATGTGATGATTGTAAACAGAGGAGATACAAT

> sgRNA to repress the initiation of sfGFP translation (variant with 2x Cas13a motifs):

AGAGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACCTCACGGATCCCATCGGGTATTTCTCCTCTGATTTAGACTA
CCCCAAAAACGAAGGGGACTAAAACATCCTTAGCGAAAGCTAAGGATTTTTTTT

> sgRNA to repress the initiation of sfGFP translation (variant with HDV ribozyme):

GATTTAGACTACCCCAAAAACGAAGGGGACTAAAACCTCACGGATCCCATCGGGTATTTCTCCTCTGGCCGGCATGGTCC
CAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCGAATGGGACATCCTTAGCGAAAGCTAAGGATTTTT
TTT

> sgRNA to repress the initiation of sfGFP translation (variant without 3’ processing):

GATTTAGACTACCCCAAAAACGAAGGGGACTAAAACCTCACGGATCCCATCGGGTATTTCTCCTCTATCCTTAGCGAAAG
CTAAGGATTTTTTTT

> Spacer of a non-targeting sgRNA (random sequence; from the variant with 2x Cas13a motifs):

GTATAATTCGAGTCCAACATAGGCGTAAAC

> Spacer of the sgRNA to repress the initiation of sfGFP translation (mutant 1; from the variant with 2x Cas13a motifs):

CTCACGGATCCCATCGGGTATTTCTCGTCT

> Spacer of the sgRNA to repress the initiation of sfGFP translation (mutant 2; from the variant with 2x Cas13a motifs):

CTCACGGATCCCATCCGGTATTTCTCCTCT

> Spacer of the sgRNA to repress the initiation of sfGFP translation (mutant 3; from the variant with 2x Cas13a motifs):

CTGACGGATCCCATCGGGTATTTCTCCTCT

> Spacer of the sgRNA to repress the initiation of sfGFP translation (mutant 4; from the variant with 2x Cas13a motifs):

CTGTCGGATCCCATCGGGTATTTCTCCTCT

> sgRNA to activate the initiation of sfGFP translation (variant with 2x Cas13a motifs):

AGAGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACTACAATCATCACATAATCACAATATCAATTGATTTAGACTA
CCCCAAAAACGAAGGGGACTAAAACATCCTTAGCGAAAGCTAAGGATTTTTTTT

> Control 5' UTR of the mRNA coding for sfGFP (related to activation variant):

ACTCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGACAT
GAAATGCGAAGAGGAGATACAAT

> RNA beacon (repression variant):

5'F/CAGAGGAGAAAUACCCGAUGGGAUCCGUGAGCUCCUCUG/3'Q

> RNA beacon (activation variant):

5'AAUUGAUAUUG/T-F/GAUUAUGUGAUGAUUGUAAACAGUACAAUACACAUAAUC/3'Q

> Coding region for eBFP2 (repression variant):

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAA
GTTCAGCGTGAGGGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGC
TGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGAGCCACGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAG
CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCACCTA
CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCGTCGACTTCAAGGAGG
ACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACATCTATATCATGGCCGTCAAGCAGAAGAAC
GGCATCAAGGTGAACTTCAAGATCCGCCACAACGTGGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACAC
CCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAGCCACTACCTGAGCACCCAGTCCGTGCTGAGCAAAGACCCCAACG
AGAAGCGCGATCACATGGTCCTGCTGGAGTTCCGCACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

> 5' UTR of the mRNA coding for eBFP2 (repression variant):

ATTATCAAATCCAAGTAAGGAGGACAGCT

> Coding region for eBFP2 (activation variant):

ATGATAAACGAGAACCTGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGA
CGTAAACGGCCACAAGTTCAGCGTGAGGGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATCT
GCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGAGCCACGGCGTGCAGTGCTTCGCCCGCTAC
CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAA
GGACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCG
TCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACATCTATATCATGGCC
GTCAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACGTGGAGGACGGCAGCGTGCAGCTCGCCGACCA
CTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAGCCACTACCTGAGCACCCAGTCCGTGCTGA
GCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCCGCACCGCCGCCGGGATCACTCTCGGCATGGAC
GAGCTGTACAAGTAA

> 5' UTR of the mRNA coding for eBFP2 (activation variant):

ACCATGAAATGCGTCTTATCTTATCTATCTCGTTTATCCCTGCATACAGAAACAGAGGAGATATGCA

> sgRNA to repress the initiation of eBFP2 translation (variant with 2x Cas13a motifs):

AGAGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACCGCCCTTGCTCACCATAGCTGTCCTCCTTAGATTTAGACTA
CCCCAAAAACGAAGGGGACTAAAACATCCTTAGCGAAAGCTAAGGATTTTTTTT

> sgRNA to activate the initiation of eBFP2 translation (variant with 2x Cas13a motifs):

AGAGATTTAGACTACCCCAAAAACGAAGGGGACTAAAACGTATGCAGGGATAAACGAGATAGATAAGATGATTTAGACTA
CCCCAAAAACGAAGGGGACTAAAACATCCTTAGCGAAAGCTAAGGATTTTTTTT

ADDITIONAL FIGURES

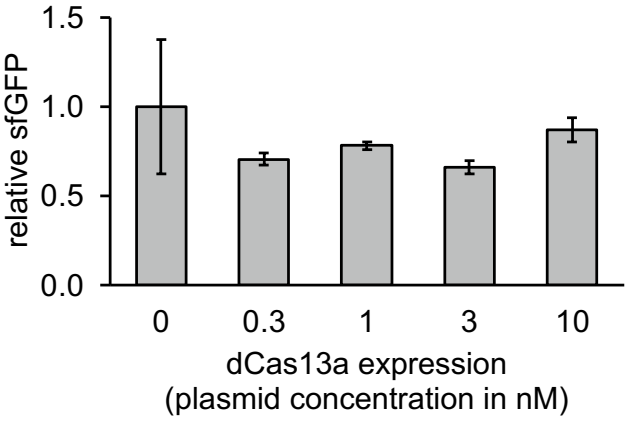

Fig. S1: Impact of dCas13a expression on sfGFP expression in the myTXTL system. The plasmid encoding the sfGFP was placed at 2 nM. Error bars correspond to standard deviations (n = 3). Statistical significance assessed by Spearman's correlation (two-tailed P = 0.91).

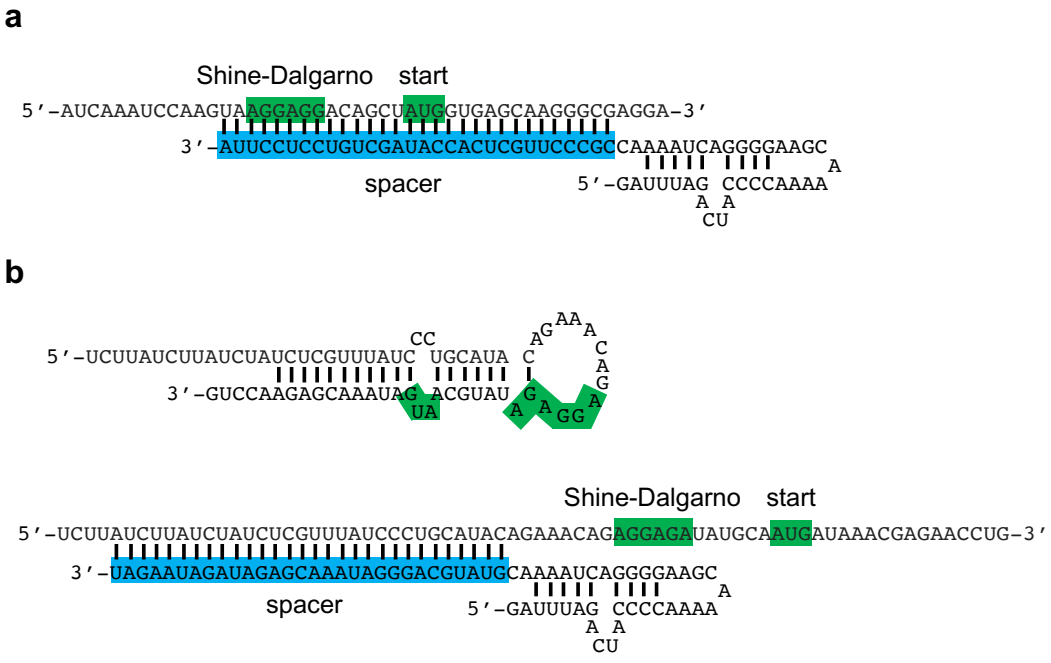

Fig. S2: RNA sequence and structure detail of the sgRNA-mRNA interaction for eBFP2 regulation. The Shine-Dalgarno box and the start codon are shown in green, while the spacer of the sgRNA is shown in blue. a) Repression. b) Activation.

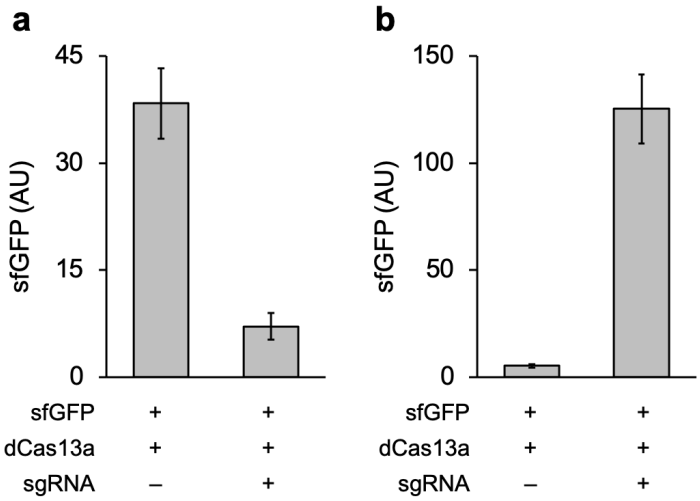

Fig. S3: a) Quantification of the green fluorescence of the colonies from Fig. 1i, the case of repression. b) Quantification of the green fluorescence of the colonies from Fig. 2h, the case of activation (*n* = 5).

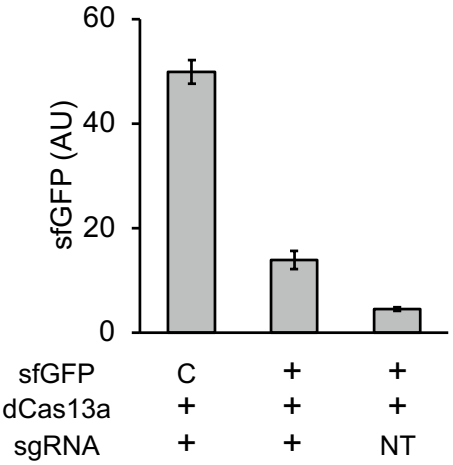

Fig. S4: Characterization of a control system without *cis*-repression of the Shine-Dalgarno box and the start codon to assess the maximal possible expression in the case of sfGFP activation. The positive control shows a 10-fold fluorescence increase with respect to the off state, while the designed system shows a 3-fold fluorescence increase through the action of the CRISPR-Cas13 ribonucleoprotein.