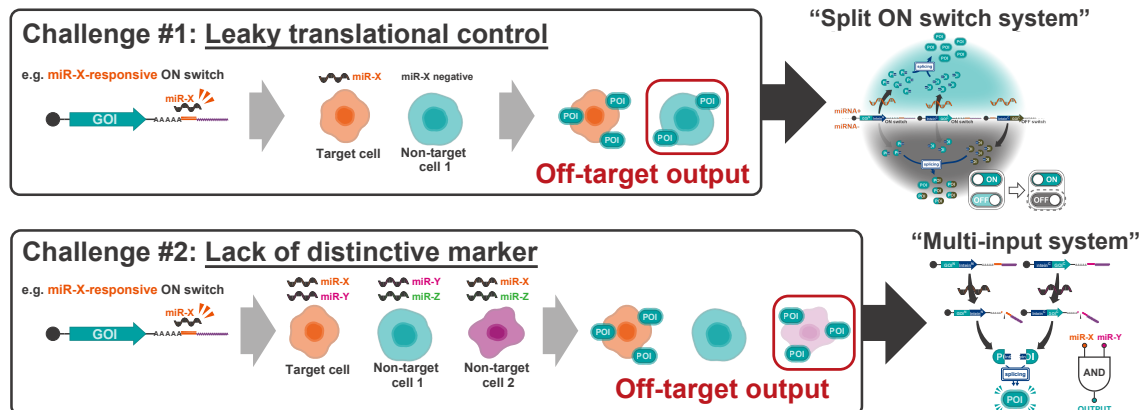




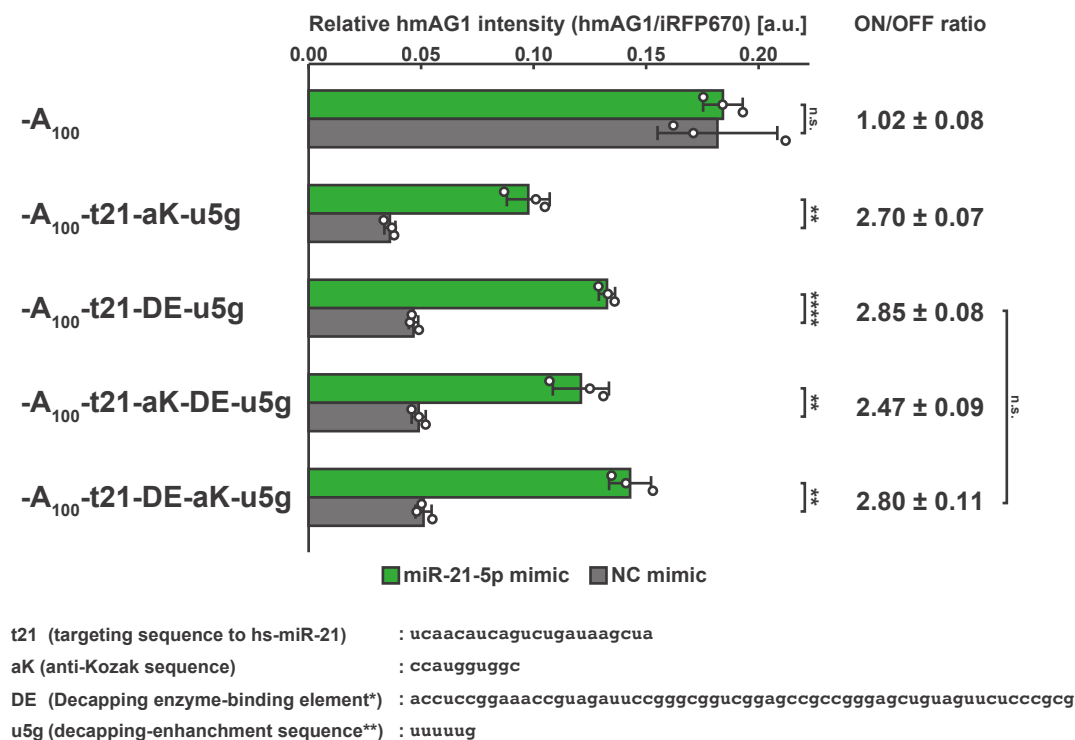
Supplementary Figure 1: Split RNA switch system can overcome two challenges that impair target cell specificity of RNA switch.

In general, translational control by a single RNA switch that targets one biomolecule (e.g., miRNA or protein) is insufficient for achieving high cell type-specificity due to two major challenges.

The first challenge is the undesired leaky expression of output protein in the OFF state (“leaky translational control”). To illustrate this issue, consider an experiment in which a miR-1-responsive ON switch is introduced into a mixed population of target cells (miR-1 activity positive, orange) and non-target cells (miR-1 negative, light blue) with the aim of expressing POI only in target cells. With leaky translation control, the POI is translated to some extent in non-target cells, resulting in a reduced ON/OFF ratio of the output protein level. Even in an “ideal” situation where only target cells in the population have the target miRNA activity and non-target cells have none, the leaky translation in the OFF state of the RNA switch itself prevents efficient fluorescent protein-based classification or cell fate control gene-based purification of target cells based on miRNA activity.

The second challenge is the difficulty in finding a target biomolecule whose expression level in the target cell type is so different from all other cell types that an RNA switch can clearly distinguish the cell (“lack of distinctive marker”). This issue is particularly problematic when trying to identify or select only target cells from a heterogeneous population of multiple cell types. The example at the bottom of the figure illustrates an experiment in which a miR-1-responsive ON switch is introduced into a mixed population of three cell types: target cells (miR-1 and miR-2 positive, orange), non-target cell 1 (miR-2 and miR-3 positive, light blue), and non-target cell 2 (miR-1 and miR-3 positive, purple). In this case, because miR-1 is also expressed in non-target cell 2, even if the miR-1-responsive ON switch is an “ideal” ON switch that overcomes the aforementioned “leaky translational control” challenge, inducing no POI expression in miR-1-negative cells, it is impossible to produce POI specifically in only the target cells. To achieve

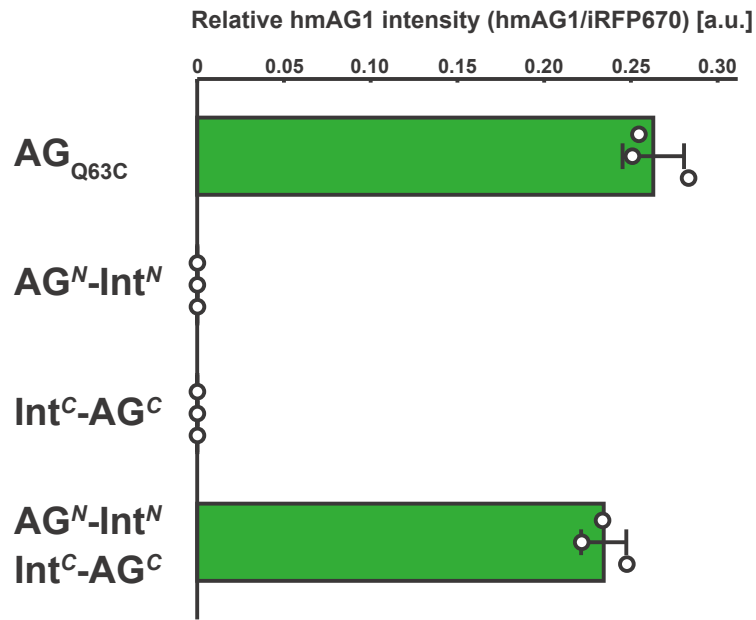
specific POI expression in target cells using a single miRNA-responsive ON switch, one must find a miRNA species with high activity exclusively in the target cells, which is difficult or impossible. The split RNA switch provides a robust solution to these two challenges. The implementation of systems such as the "split ON switch system" (Fig. 2-5) and the "split toggle-like system" (Fig. 6) using the split RNA switch can effectively address the "leaky translational control" challenge by eliminating undesired protein output in the OFF state post-translationally. In addition, the split RNA switch approach enables the construction of a "multi-input system" (Fig. 7 and 8) that incorporates multiple target miRNAs as inputs, thereby offering a powerful solution to the "lack of distinctive markers" challenge.



Supplementary Figure 2: Investigation of the extra sequence of ON switches in HEK293FT cells.

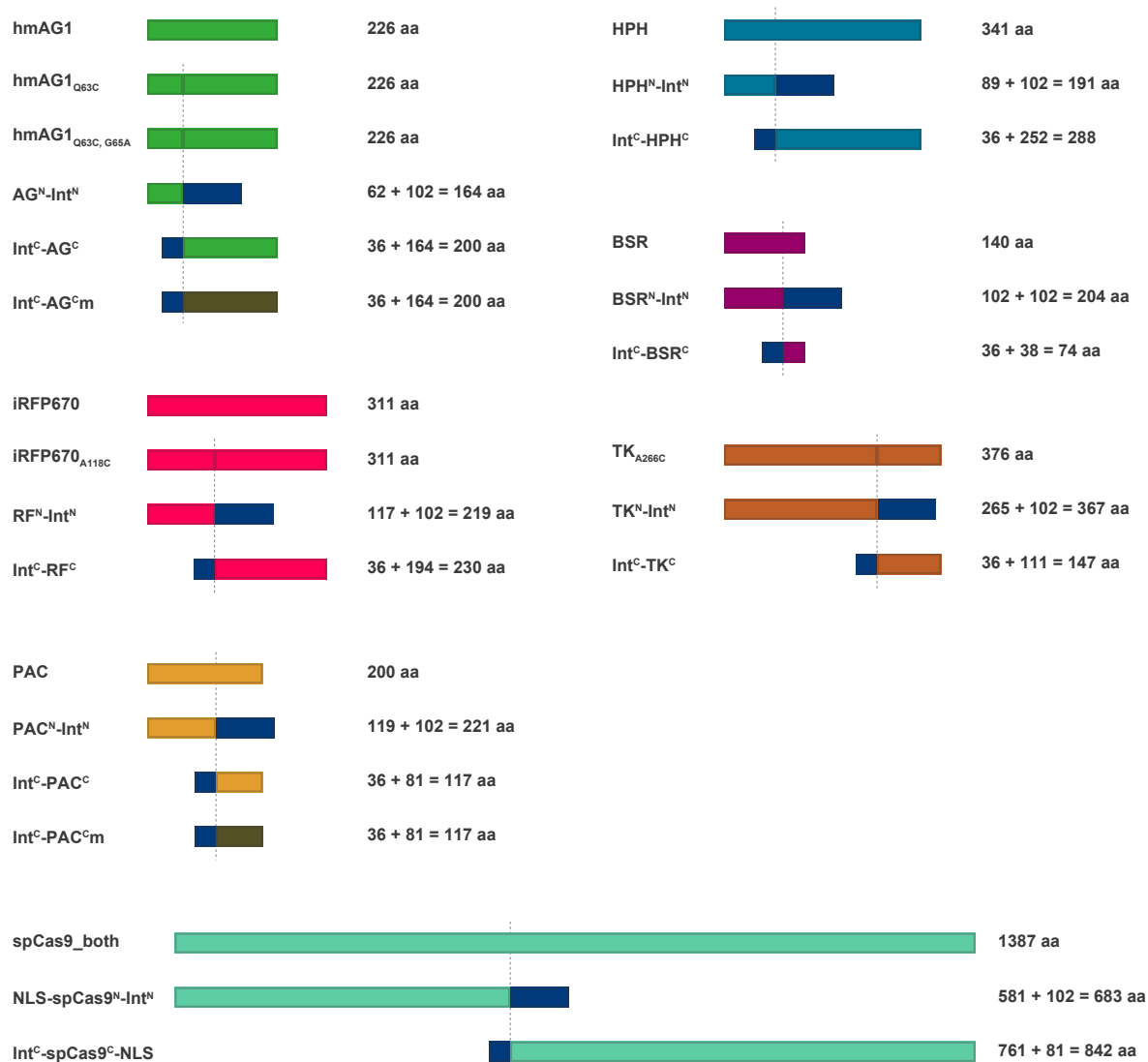
Designed miRNA-responsive ON switches with various sequences composed of “anti-Kozak” sequence and the recognition site for human Dcp2, a decapping enzyme (DE: Decapping enzyme-binding element)¹, arranged in varying orders, with u5g added at their 3’ ends as a decapping-enhancement sequence². We evaluated translation levels of each ON switch with or without the miRNA activity in a reporter assay using hmAG1 as the output and iRFP670 as a reference. The bar graph shows relative hmAG1 intensity (hmAG1/iRFP670) of HEK293FT cells treated with miR-21-5p or negative control (NC) mimics. a.u., arbitrary units. The ON/OFF ratios of all switches were below three-fold, comparable to a previous study. Error bars represent means ± SD (n=3), and data of each biological replicate are shown as a point. Statistical analysis by two-sided Welch’s *t*-test, ***P*<0.01, *****P*<0.0001, n.s.: not significant (*P*>0.05). Each *P*-value is listed in Supplementary Table 4. Source data are provided as a Source Data file.

In this study, we used the ON switch with the “-t21-DE-aK-u5g” extra sequence, which exhibited the highest translational activity in the ON state in this assay. We prioritized the ON level of the ON switch because it was initially considered that controlling the target gene with a split ON switch might reduce the ON level of protein activity.



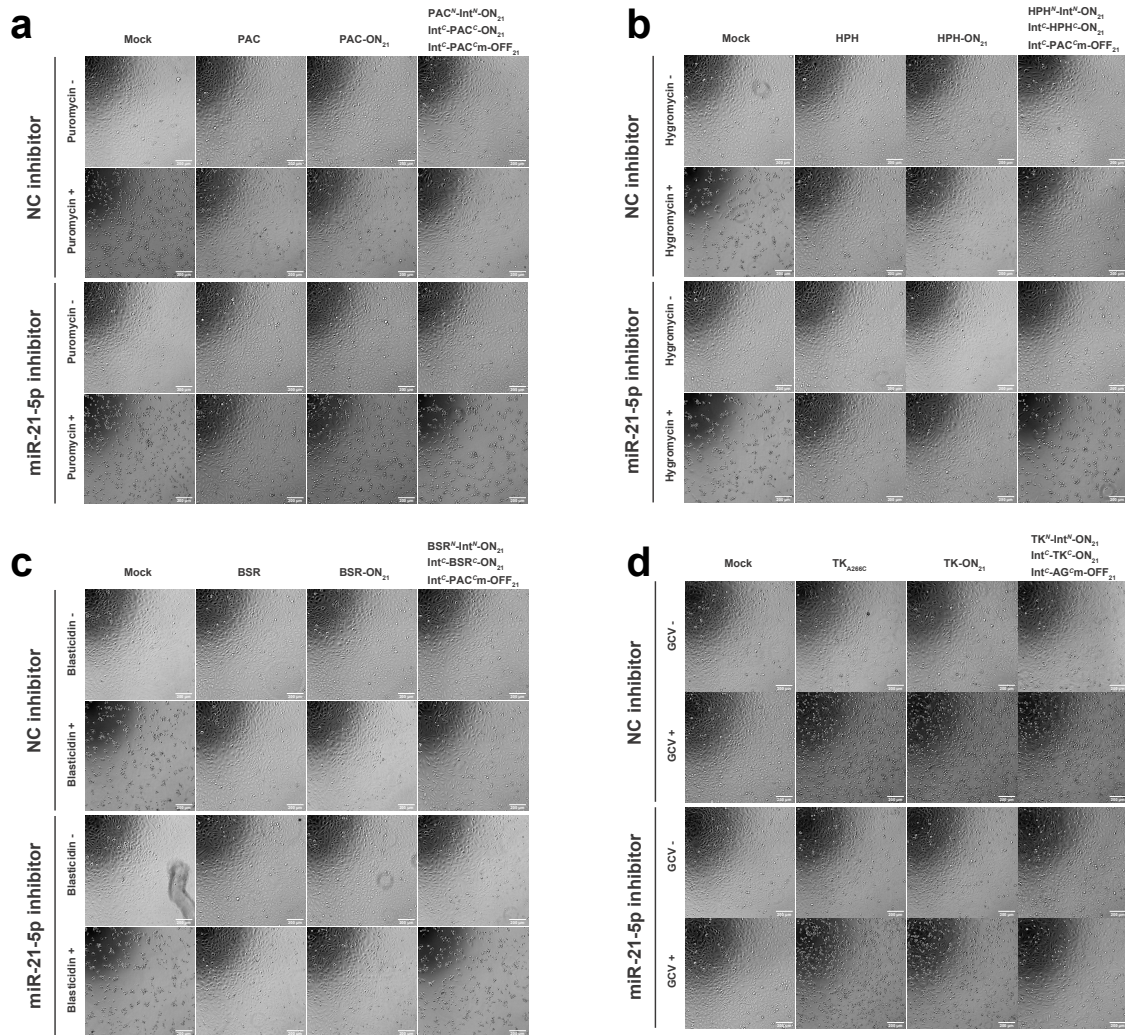
Supplementary Figure 3: Evaluation of protein splicing in HEK293FT cells.

The hmAG1 reporter assay was performed for the condition with mRNA coding protein fragments indicated on the left side of the bar graph. a.u., arbitrary units. Error bars represent means $\pm$ SD (n=3), and data of each biological replicate are shown as a point. Source data are provided as a Source Data file.



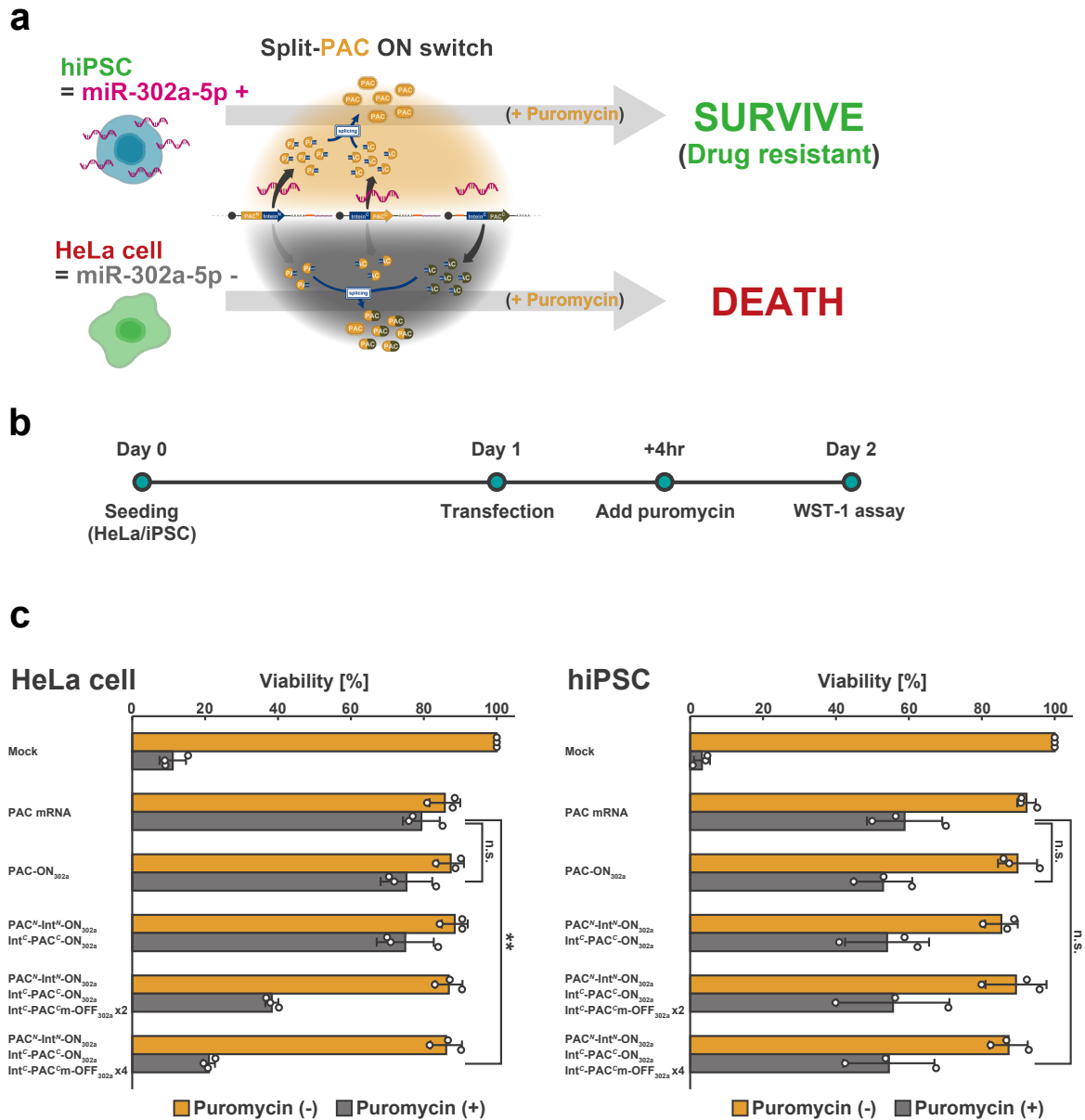
Supplementary Figure 4: The split sites of ORFs used in this research.

Schematic illustration for the split sites of the ORFs used in this research. The ORF sequence information is presented in Supplementary Table 1. The split sites of PAC, HPH, BSR, and TK are based on previous studies^{3,4}.



Supplementary Figure 5: Microscopic images of the split-PAC, -HPH, and -BSR switch system.

Representative microscopic bright-field images of HeLa cells transfected under the same conditions as shown in **Fig. 3b, d, f, h**. Scale bar, 200 μ m.



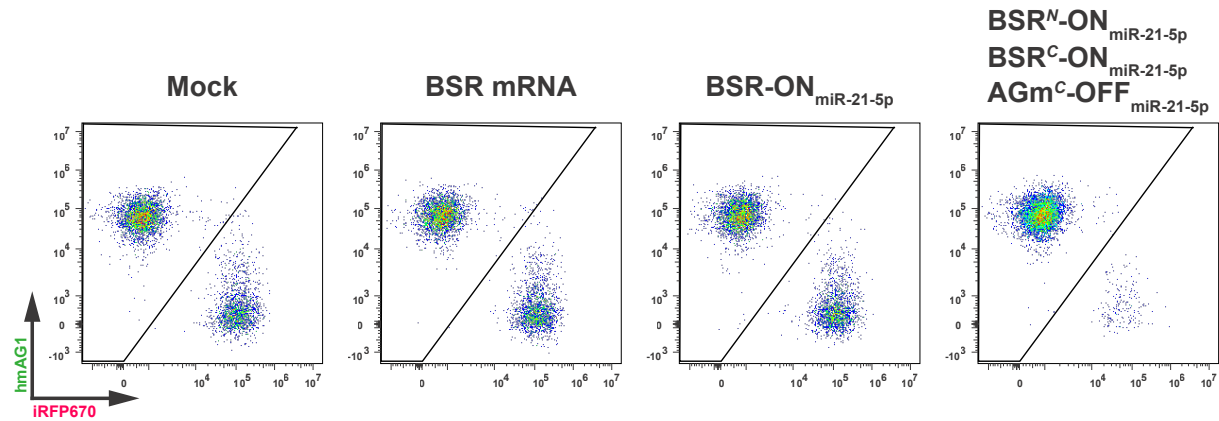
Supplementary Figure 6: miR-302a-5p responsive split-PAC ON switch system in hiPSCs and HeLa cells.

a, Schematic illustration of miR-302a-5p-responsive split ON switch system regulating puromycin N-acetyltransferase (PAC). This system suppresses the leaky activity of PAC in miR-302a-5p negative cells (HeLa cells) while maintaining the drug-resistance in miR-302a-5p positive cells (hiPSCs).

b, Schematic of the time course of this experiment.

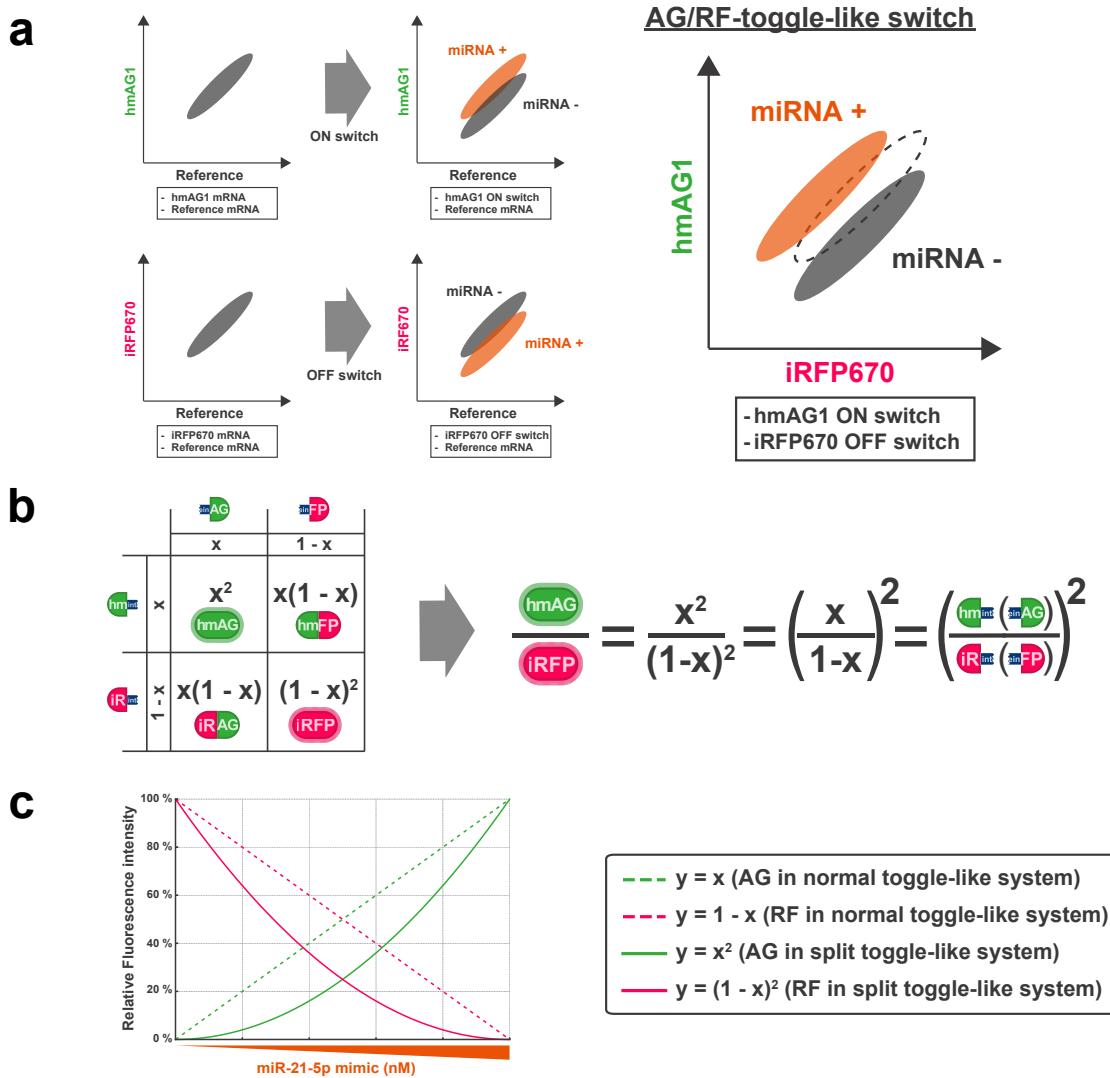
c, Viability of HeLa cells and hiPSCs in the WST-1 assay. The viability of each cell line was determined by dividing the values of each condition by those of the mock control without puromycin. Error bars represent means $\pm$ SD (n=3), and data of each biological replicate are

shown as a point. Statistical analysis by two-sided Welch's t -test, $**P<0.01$, n.s.: not significant ($P>0.05$). Each P -value is listed in Supplementary Table 4. Source data are provided as a Source Data file.



Supplementary Figure 7: Scatter plots of cells after purification by the split-BSR switch system.

Representative 2D flow cytometry plots. The horizontal axis shows the fluorescence intensity of iRFP670, and the vertical axis shows the fluorescence intensity of hmAG1.



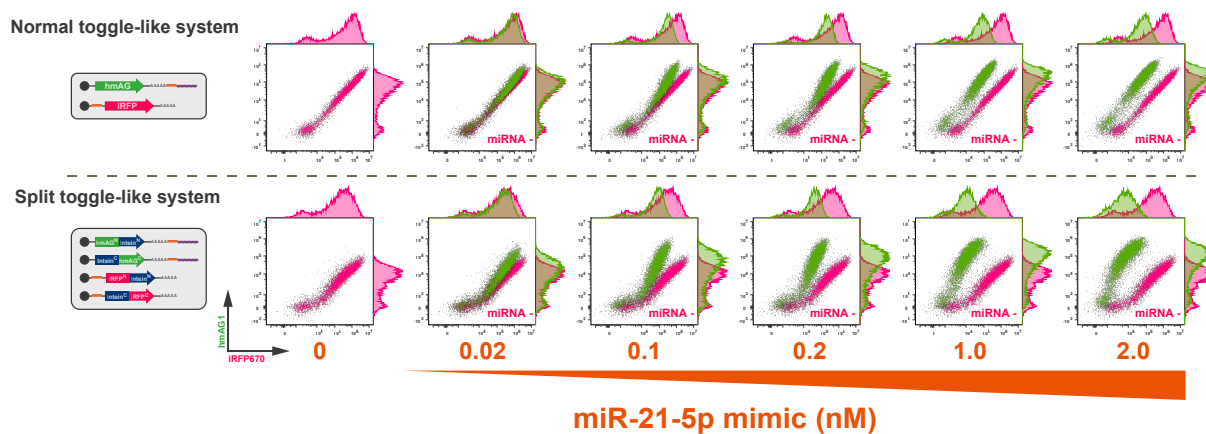
Supplementary Figure 8: Split toggle-like system shows improved binary behavior.

a, A toggle-like switch system, which switches between two fluorescences in response to target miRNA activity, can be implemented by introducing ON and OFF switches, each coding different fluorescence proteins (hmAG1 and iRFP670 in this schematic explanation). A single RNA switch often fails to completely separate two cell populations mainly due to a low ON/OFF ratio or small differences in miRNA activity. The toggle-like system enables clearer separation of two cell populations on flow cytometry 2D plots compared to the case of using a single RNA switch.

b, The expected relationship between the intracellular amount of protein fragments and the intensity of two fluorescence under the assumption that the protein splicing of N-intein and C-intein occurs randomly irrespective of the flanking extein sequences with 100% splicing efficiency.

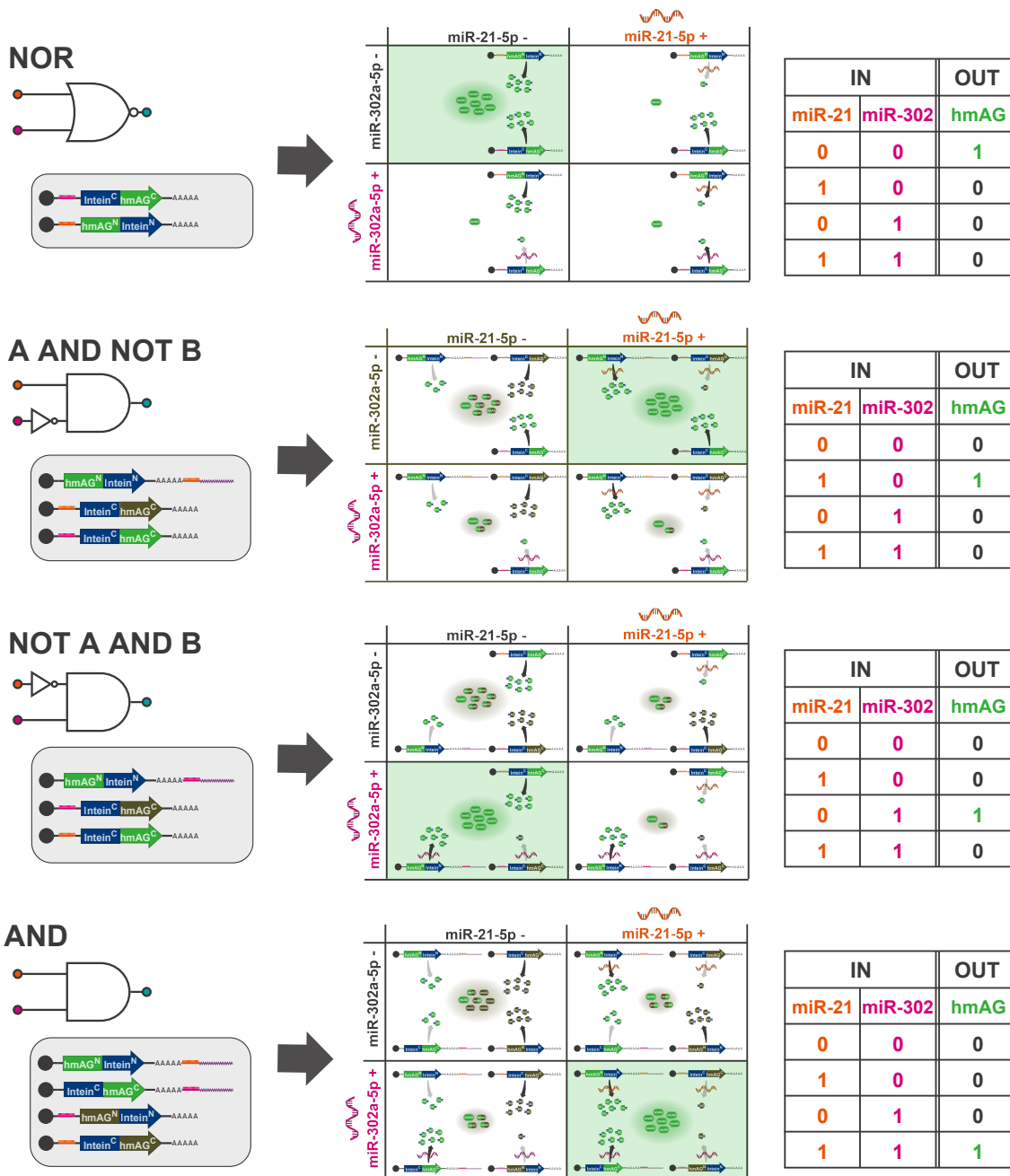
In this illustration, translation amount from the ON switch coding for hmAG1 fragments is set as x , and translation amount from the OFF switch coding for iRFP670 fragments is set as $1-x$. In the case of the "normal toggle-like system," which uses an ON switch coding full-length hmAG1 and an OFF switch coding full-length iRFP670, it is predicted that the final observed ratio of green to red fluorescence intensity will be the same as the translation ratio from the ON and OFF switches, which is $x:1-x$. On the other hand, in the "split toggle-like system," which uses split ON switches coding hmAG1 fragments and split OFF switches coding iRFP670 fragments, the ratio of the full-length protein presence will be $x^2:(1-x)^2$ as shown in this figure. This means that the fluorescence intensity ratio is the square of the actual translation ratio, allowing for a more sensitive switch between the two fluorescence in response to miRNA activity.

c, The expected relationship between miRNA concentration and the normalized intensities of hmAG1 and iRFP670 under the assumption, in addition to the assumption mentioned in **b**, that the AG-ON and RF-OFF switches modulate the translation of downstream genes in proportion to the miRNA concentration and that the translation output from these switches reach 100% and 0% respectively at a certain threshold value.



Supplementary Figure 9: Scatter plots of cells introduced with normal or split toggle-like systems.

Representative 2D flow cytometry plots. The horizontal axis shows the fluorescence intensity of iRFP670, and the vertical axis shows the fluorescence intensity of hmAG1. The plots for the cell populations without miR-21-5p mimic (far left, magenta) are shown alongside the results of each condition, with varying concentrations of miR-21-5p mimic (green).



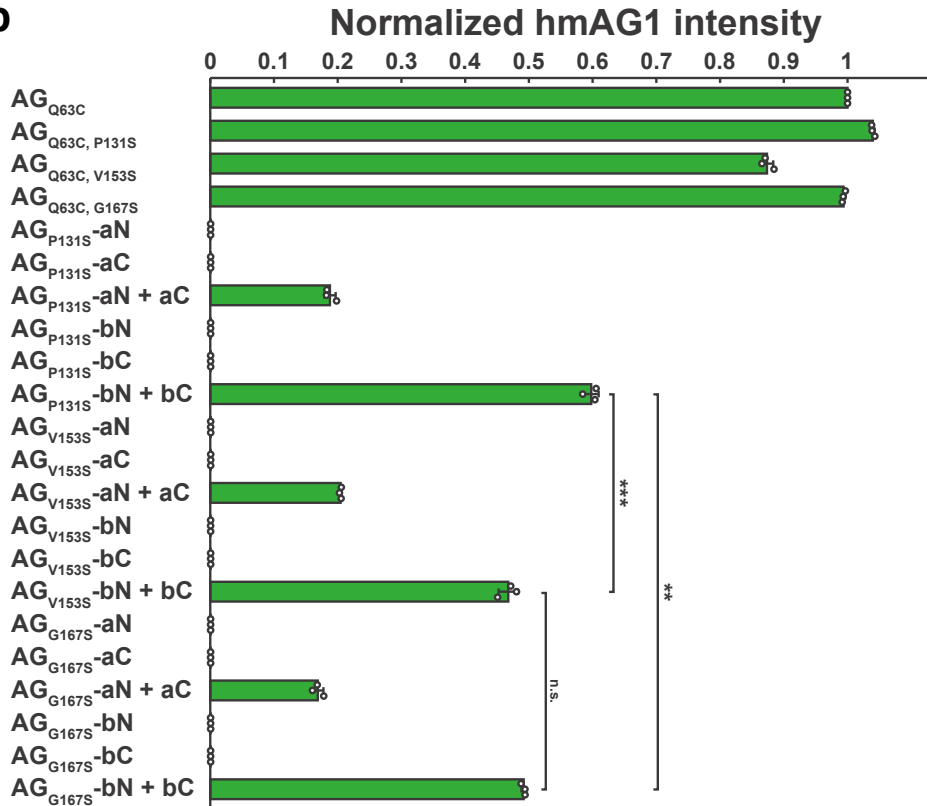
Supplementary Figure 10: Mechanism of four types of two-input systems.

Schematic illustrations of the expected behaviors of each RNA switch set in transfected cells. Introducing an ON switch along with a complementary OFF switch, coding the opposing inactivated fragment but sharing the same miRNA target site, allows for the suppression of the logic gate leakage.

a



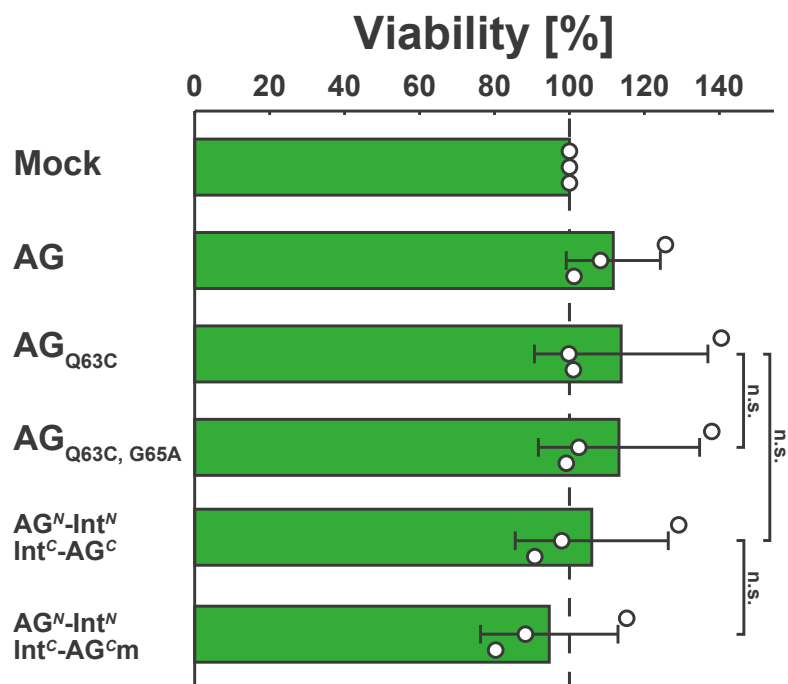
b



Supplementary Figure 11: Evaluation of split sites of hmAG1 and SspDnaB intein for the construction of three-input logic circuits.

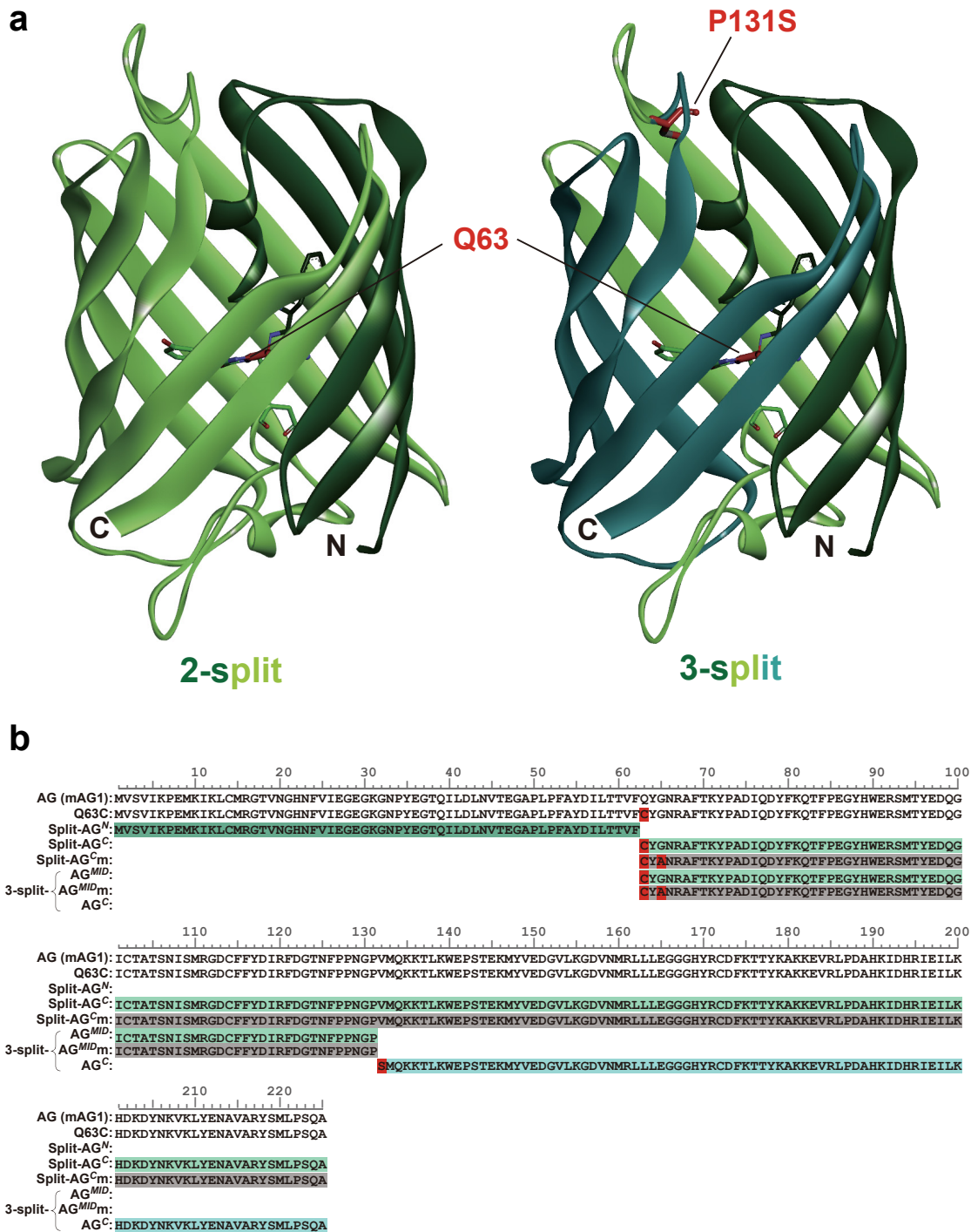
a, In this study, to construct miRNA-responsive three-input logic circuits using hmAG1 as the output protein, we explored (i) a second split site within hmAG1 in addition to the first split site (62F/63Q) and (ii) a split site within the split-intein, SspDnaB_{M86}, which is known to be orthogonal to NpuDnaE. For (i), we evaluated three split patterns: 130G/131P, 152G/153V, and 166G/167G, while for (ii), we assessed two split patterns: 11A/12S ("type-a") and 105Q/106L ("type-b"). To evaluate all possible six combinations, we designed a total of 12 fragmented variants and measured the fluorescence activity when introducing either the N- or C-terminal fragments alone, or both fragments together, in HEK293FT cells.

b, Normalized fluorescence intensity in HEK293FT cells transfected with mRNA coding full-length or fragmented hmAG1 variants. Error bars represent means $\pm$ SD (n=3), and data of each biological replicate are shown as a point. Statistical analysis by two-sided Welch's *t*-test, ** $P < 0.01$, *** $P < 0.001$, n.s.: not significant ($P > 0.05$). Each *P*-value is listed in Supplementary Table 4. Source data are provided as a Source Data file.



Supplementary Figure 12: Evaluation of cytotoxicity of split-intein and mutated protein fragments in HEK293FT cell.

Viability of HeLa cells transfected with each set of mRNAs in the CellTiter-Glo Luminescent Cell Viability Assay. The viability of each cell line was determined by dividing the values of each condition by those of the mock control. Error bars represent means $\pm$ SD ($n=3$), and data of each biological replicate are shown as a point. Statistical analysis by two-sided Welch's t -test, n.s.: not significant ($P>0.05$). Each P -value is listed in Supplementary Table 4. Source data are provided as a Source Data file.

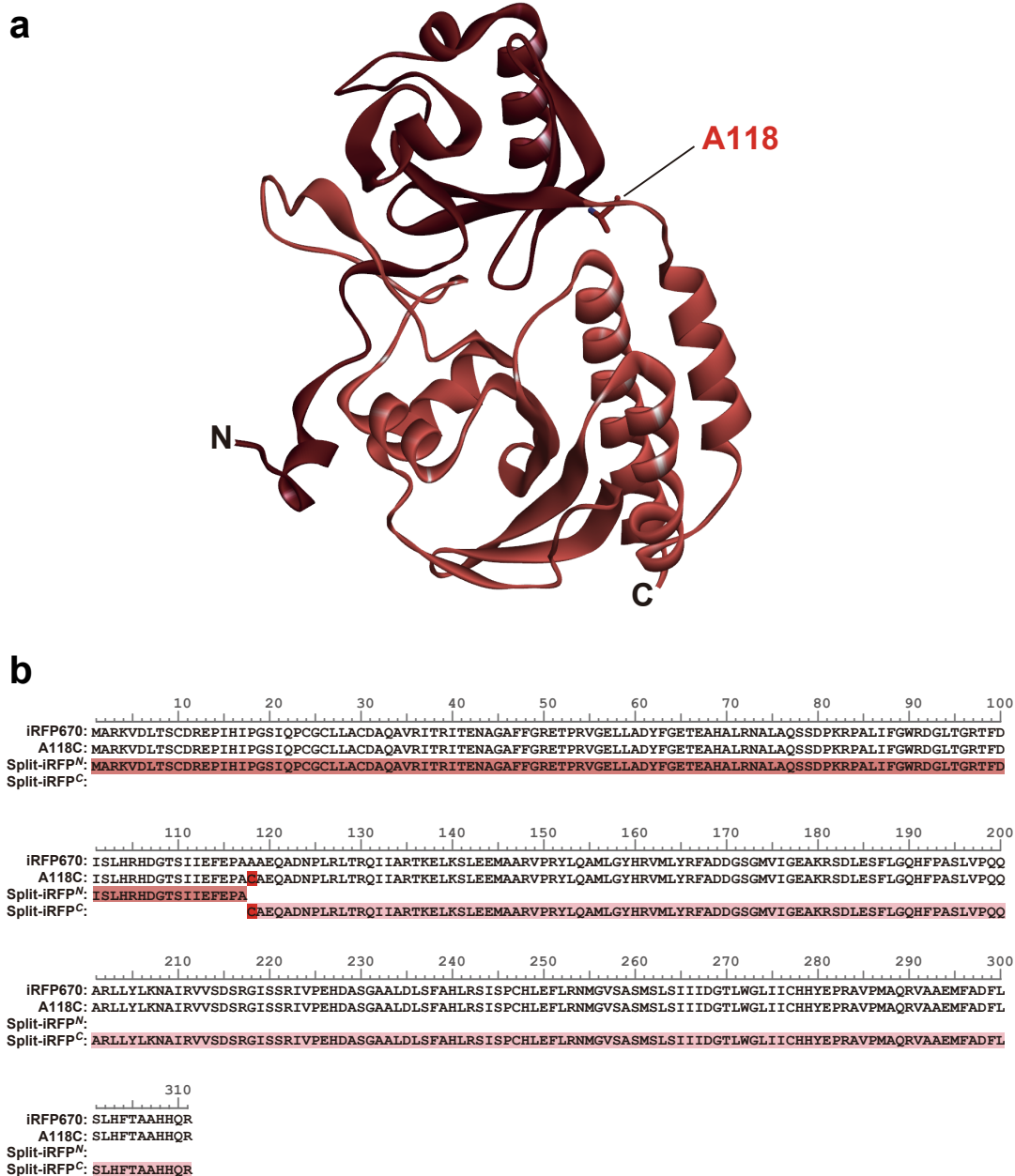


Supplementary Figure 13: Split points for monomeric AG.

a, Three-dimensional structure of AG (mAG, mAG1: monomeric Azami-Green (1); Protein Data Bank ID: 3adf). Dark green: split-AG^N (common for two-split (left) and three-split (right)), light

green: split-AG^C for two-split (left) and AG^{MID} for three-split (right), blue green: AG^{MID} for three-split (right).

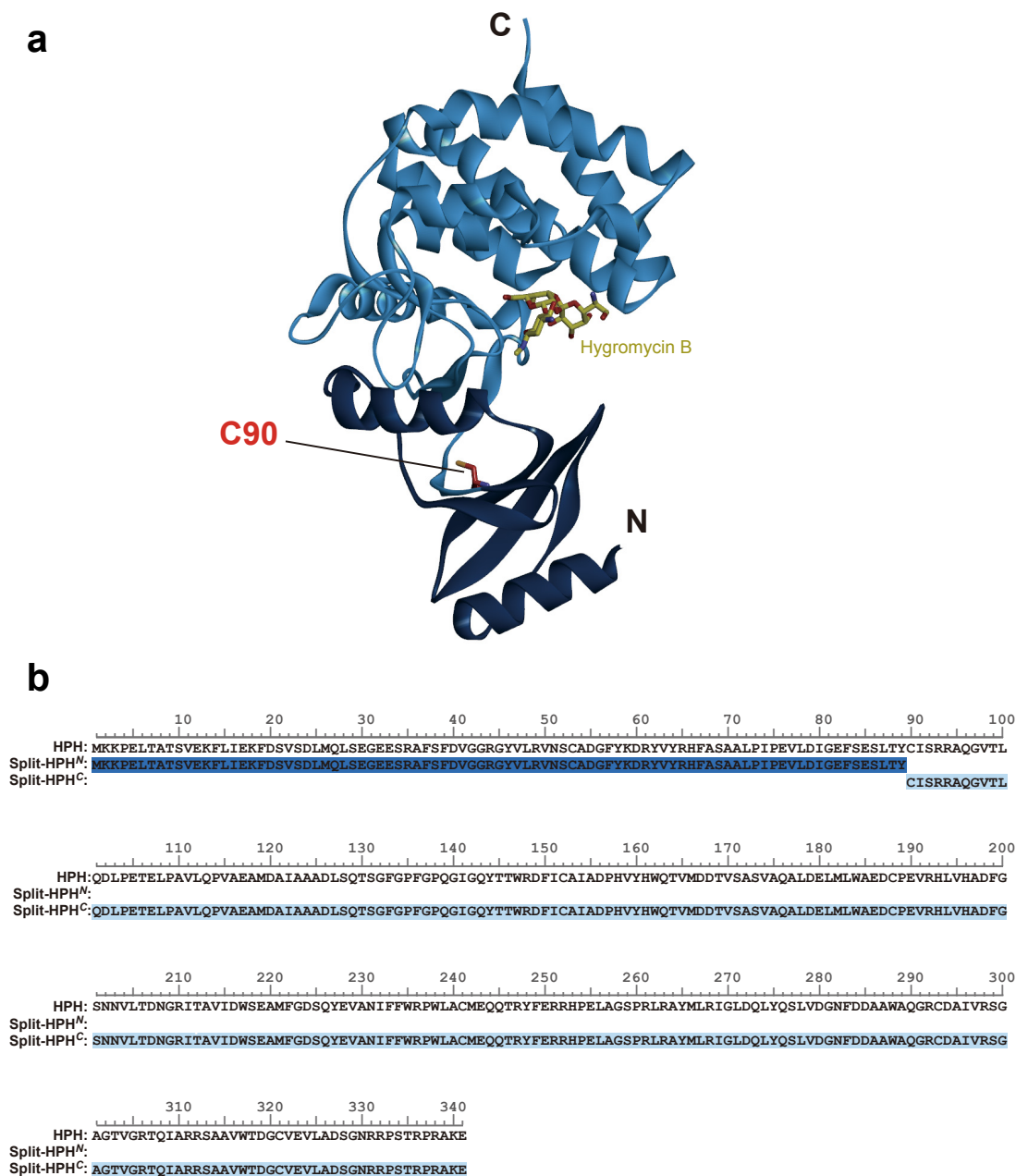
b, Amino acid sequences of the original AG, Q63C mutant, split-AG^N, split-AG^C, split-AG^Cm (leak-canceler), three-split-AG^{MID}, three-split-AG^{MID}m (leak canceler in three-split experiments), and three-split-AG^C. Q63C mutant corresponds to intein-spliced mature protein, which has the cysteine for the intein-splicing. Splitting position was designed referring to another fluorescent protein, GFP⁵. In split-AG^Cm, the G65A mutant, corresponding to G67A in GFP, was used as a leak-canceller to produce a non-functional fragment to inhibit the formation of a functional fluorophore⁶.



Supplementary Figure 14: Split points for RF.

a, Three-dimensional structure of RF (iRFP670) predicted by AlphaFold2. Dark red: split-iRFP^N, light red: split-iRFP^C.

b, Amino acid sequences of the original iRFP670, A118C mutant, split-iRFP^N, and split-iRFP^C. A118C mutant corresponds to intein-spliced mature protein, which has the cysteine for intein-splicing. The splitting position was designed within the loop between PAS and GAF domain⁷.

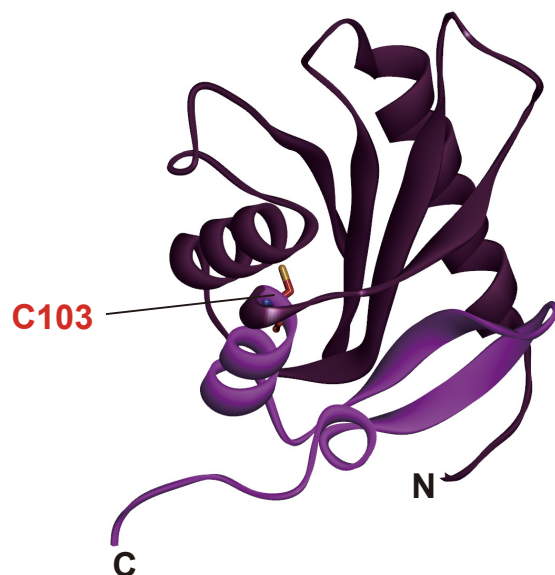


Supplementary Figure 15: Split points for HPH.

a, Three-dimensional structure of HPH (Hygromycin-B 4-O-kinase; Protein Data Bank ID: 3tyk). Dark blue: split-HPH^N, light blue: split-HPH^C.

b, Amino acid sequences of the original HPH (derived from *Escherichia coli* Uniprot: P00557), split-HPH^N, and split-HPH^C. Splitting position was designed by referring to ref³.

a



b

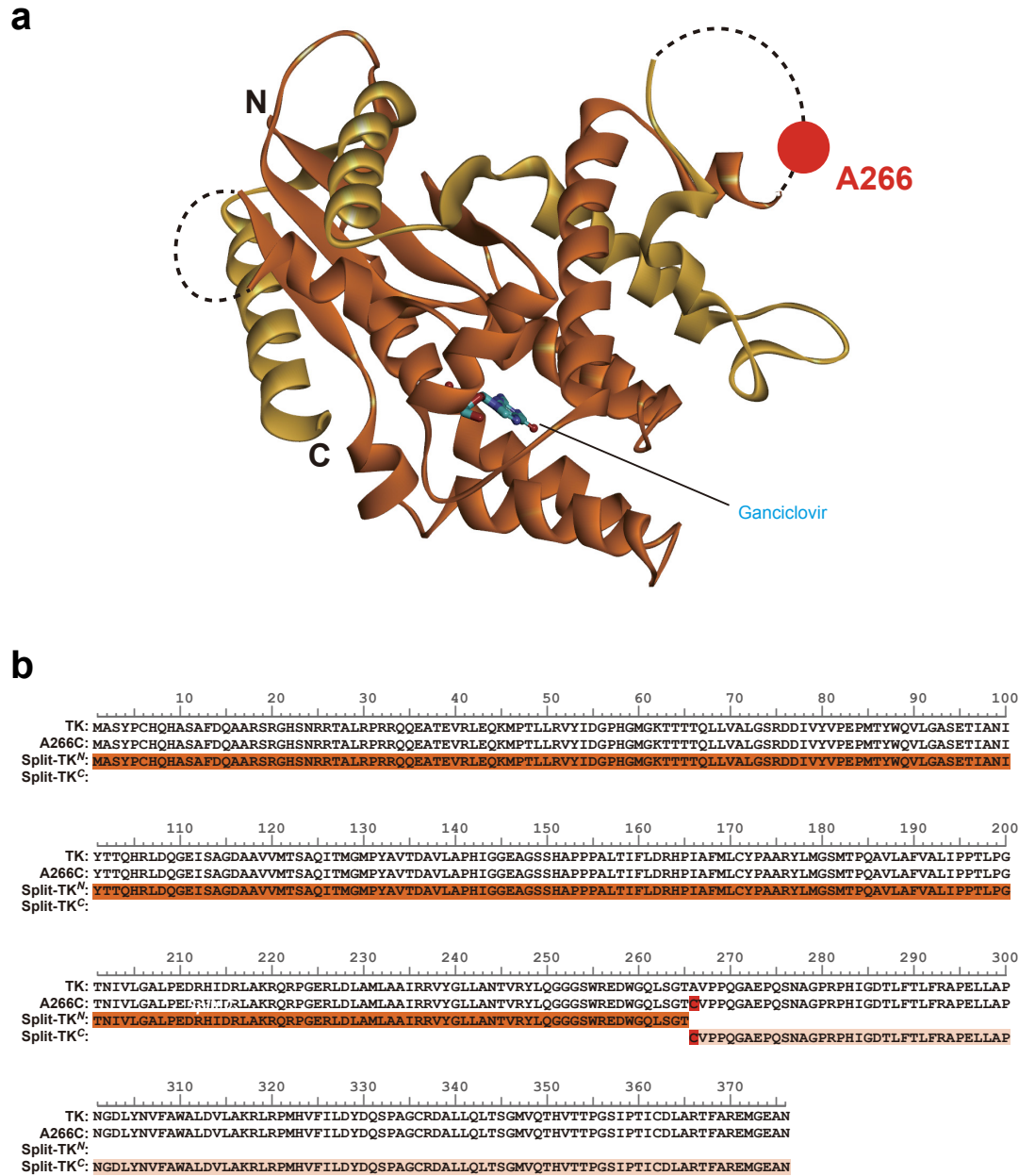
BSR: MKTFNISQDLELVEVATEKITMLYEDNKHVGAAIRTKTGEIISAVHIEAYIGRVTVC AEAIAGSAVSNQKDFDTIVAVRHPYSDEVDRSIRVVSPC
 Split-BSR^N: MKTFNISQDLELVEVATEKITMLYEDNKHVGAAIRTKTGEIISAVHIEAYIGRVTVC AEAIAGSAVSNQKDFDTIVAVRHPYSDEVDRSIRVVSPC
 Split-BSR^C: MKTFNISQDLELVEVATEKITMLYEDNKHVGAAIRTKTGEIISAVHIEAYIGRVTVC AEAIAGSAVSNQKDFDTIVAVRHPYSDEVDRSIRVVSPC

BSR: GMCRELISDYAPDCFVLIEMNGKLVKTTEEELIPLKYTRN
 Split-BSR^N: GMCRELISDYAPDCFVLIEMNGKLVKTTEEELIPLKYTRN
 Split-BSR^C: GMCRELISDYAPDCFVLIEMNGKLVKTTEEELIPLKYTRN

Supplementary Figure 16: Split points for BSR.

a, Three-dimensional structure of BSR (Blasticidin-S deaminase) predicted by AlphaFold (AlphaFold Protein Structure Database: AF-P33967-F1-model_v4). Dark purple: split-BSR^N, light purple: split-BSR^C.

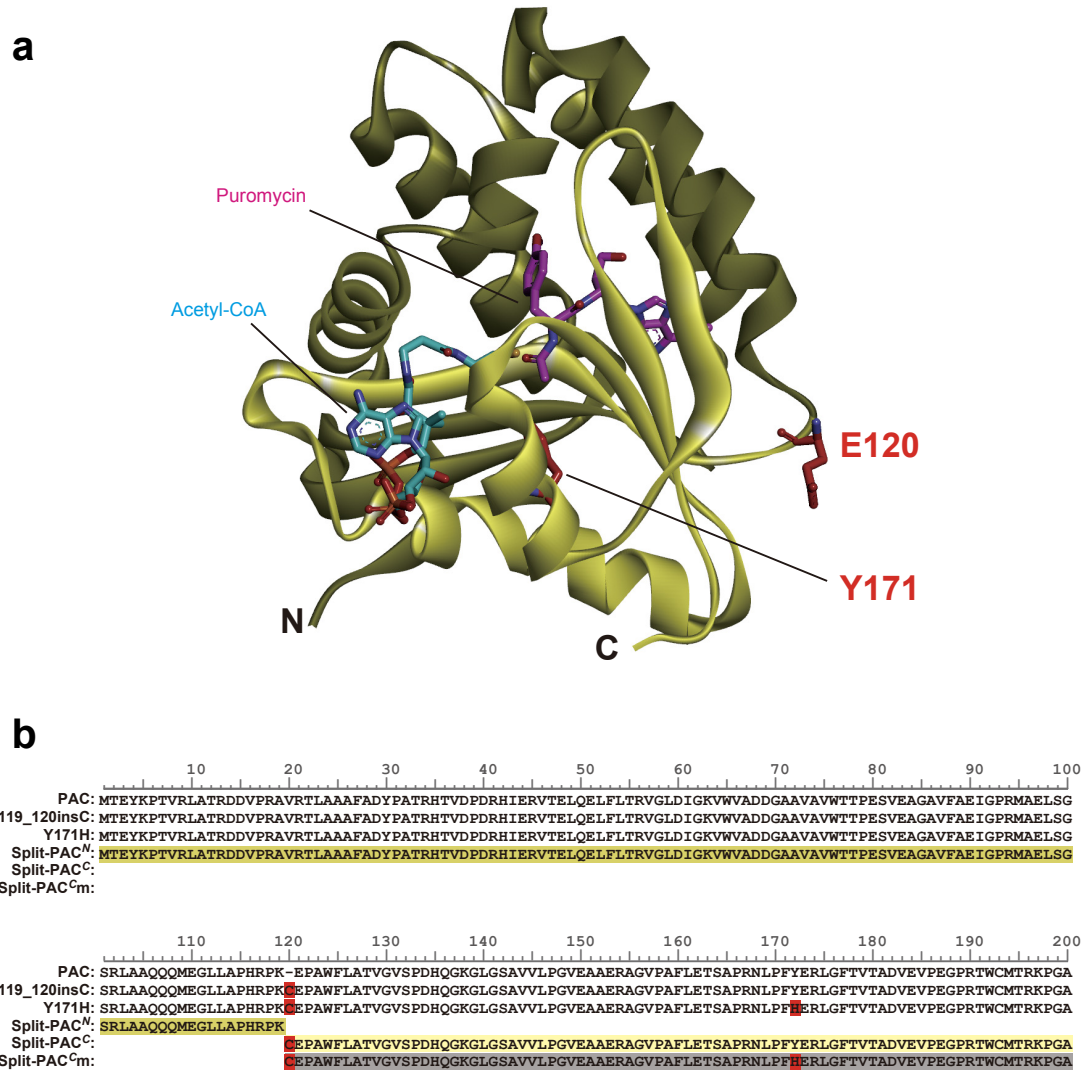
b, Amino acid sequences of the original BSR (derived from *Bacillus cereus*; UniProt: P33967), split-BSR^N, and split-BSR^C. Splitting position was designed by referring to ref³.



Supplementary Figure 17: Split points for TK.

a, Three-dimensional structure of TK (Thymidine kinase, Protein Data Bank ID: 1ki2). Dark orange: split-TK^N, light orange: split-TK^C.

b, Amino acid sequences of the original TK (derived from HSV: Human herpes simplex virus 1, Gene ID: 24271467), split-TK^N, split-TK^C. Splitting position was designed by referring to ref⁴.

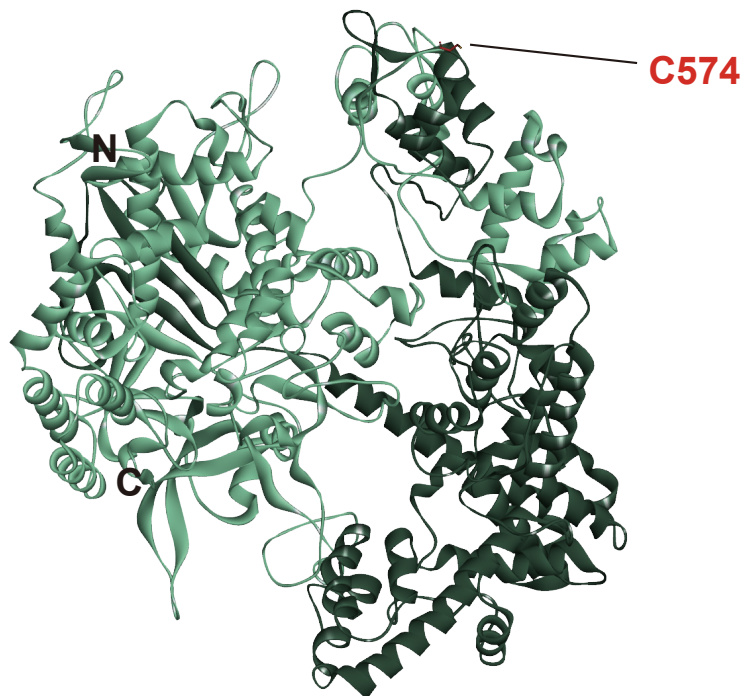


Supplementary Figure 18: Split points for PAC.

a, Three-dimensional structure of PAC (Puromycin-N-acetyltransferase; Protein Data Bank ID: 7k0a). Dark yellow: split-PAC^N, light yellow: split-PAC^C.

b, Amino acid sequences of the original PAC (derived from *Streptomyces alboniger*, UniProt AC: P13249), 119_120insC mutant, Y171H mutant, split-PAC^N, split-PAC^C, and split-PAC^{Cm}. 119_120insC mutant corresponds to intein-spliced mature protein, which has a cysteine insertion between K119 and E120. Y171H is a defective mutation⁸, used in split-PAC^{Cm} as a leak-canceller to produce a non-function PAC protein. Splitting position was designed by referring to ref³.

a



b

10 20 30 40 50 60 70 80 90 100 110 120

Cas9 : MDKKYSIGLDIGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGETAETRLKRTARRRYTRKRNRCYLQEIFSNEMAKVDDSFHRLLESFLVEEDKKHERHPFG
 Cas9^N: MDKKYSIGLDIGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGETAETRLKRTARRRYTRKRNRCYLQEIFSNEMAKVDDSFHRLLESFLVEEDKKHERHPFG
 Cas9^C: MDKKYSIGLDIGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGETAETRLKRTARRRYTRKRNRCYLQEIFSNEMAKVDDSFHRLLESFLVEEDKKHERHPFG

130 140 150 160 170 180 190 200 210 220 230 240

Cas9 : NIVDEVAYHEKYPTIYHLRKKLVDSKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVVKLFQIQVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNLFGN
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 Cas9^C: NIVDEVAYHEKYPTIYHLRKKLVDSKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVVKLFQIQVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNLFGN

250 260 270 280 290 300 310 320 330 340 350 360

Cas9 : LIALSLGLTPNFKSNFDLAEDAKLQSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYA
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370 380 390 400 410 420 430 440 450 460 470 480

Cas9 : GYIDGASQSEFYKPIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYVVGPLARGNSRFAMTRKSEETITPWNFEE
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490 500 510 520 530 540 550 560 570 580 590 600

Cas9 : VVDKGASQSFIERMTNFDKNLPNEKVLPHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNKRVTVKQLKEDYFKIECFDSVEISGVEDRFNASLGTYHDLKI
 Cas9^N: VVDKGASQSFIERMTNFDKNLPNEKVLPHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNKRVTVKQLKEDYFKIECFDSVEISGVEDRFNASLGTYHDLKI
 Cas9^C: VVDKGASQSFIERMTNFDKNLPNEKVLPHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNKRVTVKQLKEDYFKIECFDSVEISGVEDRFNASLGTYHDLKI

610 620 630 640 650 660 670 680 690 700 710 720

Cas9 : IKDKDFLDNEENEDILEDIVLTITLTFEDREMIEERLKYTAHLFDDKVMQKLRRTYTGWGLSRKLINGIRDKQSGKTITLDFLKSDGFANRNFQLIHDDSLTFKEDIQKAQVSGQDLSL
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730 740 750 760 770 780 790 800 810 820 830 840

Cas9 : HEHIANLAGSPAIIKGIQTQVVDDELVKVMGRHKPENIVEMARENQTTQKGQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYVDVH
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 Cas9^C: HEHIANLAGSPAIIKGIQTQVVDDELVKVMGRHKPENIVEMARENQTTQKGQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYVDVH

850 860 870 880 890 900 910 920 930 940 950 960

Cas9 : IVPQSFLKDDSIDNKVLTSDKNRGSNDVPSEEVVKMKNYRQLNAKLITQRFQDNLTKAERGLSELDKAGFIKRLVETQITKHVAQILD SRMNTKYDENDKLIREVKVITLKS
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970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080

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1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200

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 Cas9^N: ATVRKVLSPQVNIKKTEVQTTGGFSKESILPKRNSDKLIARKKDWDPKKYGGDFSPTVAYSVLVAVKVEGKSKKLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIILKLPK
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1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320

Cas9 : YSLFELENGKRMLASAGELQGNELALPSKYVNFYLYASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL SAYNKHHRDKPIREQAENIIHLFTLTNLGA
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1330 1340 1350 1360

Cas9 : PAAFKYFTTIDRKRYTSKEVLDATLIHQSTIGLYETRIDLSQLGGD
 Cas9^N: PAAFKYFTTIDRKRYTSKEVLDATLIHQSTIGLYETRIDLSQLGGD
 Cas9^C: PAAFKYFTTIDRKRYTSKEVLDATLIHQSTIGLYETRIDLSQLGGD

Supplementary Figure 19: Split points for *sp*Cas9.

a, Three-dimensional structure of Cas9 (*Streptococcus pyogenes* Cas9, Protein Data Bank ID: 5f9r). Dark green: split-Cas9^N, light green: split-Cas9^C.

b, Amino acid sequences of original Cas9, split-Cas9^N, split-Cas9^C. Splitting position was designed referring to ref⁹.

Nucleotide sequence used in this study.

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iRFP670 ^{A118C}	AUGGCGGUAAGGUCGAUCUACCUCUGCGAUCGCGAGCGGAUCCACAUCCCAGGAGCAUUCAGCCGUGCGGCGGCGGUCUAGCCUGCGACGC GAGGCGGUGCGGAUACGCGCAUACGGAAAUAGCGGCGGCGUUCUUGGAGCGGAAACUCCGCGGUGCGGAGCUACUCCGCGAUUACUUCG GCGAGACCGAAGCCAUUGCGUGCGCAACGCAUCGCGCAGUCCUCCGAUCCAAAGCGACCGGCGUGAUUUCGGUUGCGCGACGCGCGUACCC GGCGGACCCUUCGACAUUCACUGCAUCGCGCAUGACGGUACAUUGCAUUCGAGUUCGAGCCUGCGUGCGGCAACAGGCGGACAAUCCGCGG CGUACCGCGGAGAUACUCCGCGCACAAAGAACUGAAGUCGCGAAGAGAUAGCGCGACGGUGCGCGCUAUUCGAGCGCAUUCGCGGCU AUCACCGCGUGAUGUACCGCUUCGCGGACGACGCGUCCGGAGUGGAGUCCGCGAGCGGAGCGGACCGUACGAGAGCUUCGCGGUCAG CACUUUCCGCGGUCGUGGUCGCGCAGCGCGCGGCUACUGUACUUGAAGAACCGGAUCCGCGUGGUCUCCGGAUUCGCGCGGCAUCAGCAGCGG GAUCGUGCGCGAGCACGCGCUCGCGCGCGCGCGUCGAGUUCGCGGACCGUGCGAGCAUUCGCGCGGCAUCUGGAAUUCUGCGGA ACAUGGCGGUCAGCGCCUCGAGUUCGCGUGCGAUCAUUAGAGCGGACGCUAUGGGGAUUGAUCUUCGCAUCAUACAGCGCGGUGCGGUG CCGAUGGCGCGAGCGCGCGGCGGAAAUUGUUCGCGGACUUCUAUCGUGGACUUCACCGCGCGCCACCAACGCGuga	936	311
RF ^N -Int ^N	AUGGCGGUAAGGUCGAUCUACCUCUGCGAUCGCGAGCGGAUCCACAUCCCAGGAGCAUUCAGCCGUGCGGCGGCGGUCUAGCCUGCGACGC GAGGCGGUGCGGAUACGCGCAUACGGAAAUAGCGGCGGCGUUCUUGGAGCGGAAACUCCGCGGUGCGGAGCUACUCCGCGAUUACUUCG GCGAGACCGAAGCCAUUGCGUGCGCAACGCAUCGCGCAGUCCUCCGAUCCAAAGCGACCGGCGUGAUUUCGGUUGCGCGACGCGCGUACCC GGCGGACCCUUCGACAUUCACUGCAUCGCGCAUGACGGUACAUUGCAUUCGAGUUCGAGCCUGCGUGCGUGAGCUACGAGACAGAGAUCCUGAC CGUGGAAUACGCGCGUGCGCUAUCGCGAAGAUUCGUGGAAAGCGGAUUCGAGUGCAGCGGUGUACUCCGUGGAAUACACGGCAACAUUCACCC AGCCUUGGCGUACUGGCGACGACAGAGGCGAGCAAGAGGUGUUCGAGUACUGCGUGGAAGUUGGAGCGGUGAUCAGAGCCACCAAGGACCAAG UUAUGACAGUGGACGCGCAGAUUGCGCCAUUGACGAGAGAUUCUGAGCGGAGCUGGACCUUGAUGAGAGUGGACAACCGCCUUAACuga	660	219
Int ^C -RF ^C	AUGAUCAGAUCGCCACAGGAAGUACUGGGCAAGCAGAACGUGUACGACAUUGCGGUGGAAACGGGACCAACAUCUGCCUGAAGAACGGCUU UAUCCGCGAGCAACUGCGGAAACAGGCGGACAAUCCGCGCGGCGUACGCGCGGAGAUACGCGCGCACCAAGAACUGAAGUCGUCGGAAGAGA UGGCGCGACGCGGUGCGCGCUAUCUGCAGGCGAUCGUGCGUAUACCGCGUGAUGUUGUACCGCUUCGCGGAGCAGCGGCUCCGGGAUGGUGAU GGCGAGGCGAAGCGCAGCGACUUCGAGAGCUUCUCCGGUCAGCACUUCUCCGCGGUCGUGGUGCCGAGCAGCGCGCGGCUACUGUACUUGAAGAA CGCGAUCCGCGUGGUCUGGAAUUCGCGCGGCAUCAGCAGCGGGAUUCGUGCGCGAGCAGCGCCUCCGCGCGCGCGUCGUAUCUGUGUUCGCGC ACCUCCGCGAGAUUCGCGCCUGCCAUUCGAAUUCUUGCGGAACAUGGGCGUGCAGCGCCUGAUGUGCGUGUGCAUCAUUGAGCGGACGCUA UGGGGAUUGAUCAUUCUGCAUCAUACGAGCGCGGUGCGGCGAUGGCGCAGCGGUGCGGCGGAAUUGUUCGCGGACUUCUUAUCGUGCA CUUACCGCGCGCCACCAACGCGuga	693	230
PAC	AUGACCGAGUACAAAGCUACAGUGCGGUGGCGACAGAGAUAGUGCCUAGAGCGGUCAGAAACACUGGCGCGUGCCUUCGCGGAUUAUCCUGC CACAAGACACACCGUGGAUCCGACAGACACAUCGAGAGAGUGACCGAGCUGCAAGAGCUUUCUGACAGAGUGCGGCGUGGACAUUGCGGAAAG UGUGGGUUGCAGAUAGUGCGCGCGUGGCGUGUGGACAAACCCUGAAUUCUGGGAAGCGCGCGUGUGUUCGCGGAGUCCGAGCUAGAAUG GCGGAGCUGUGCGGUCUAGAGUGGCGUGCAGCAGCAGAUUGGAAGGACUGUGGCGCCUACAGACCUAAGUGCGAGCGCGCGUGUUCUGC UACCGUGGGAGUGUCCCGUACCAACAGGCAAGGACUGGGAUUCUGUGGUGUGCGGCGUGGAGCGGUGAAGCGCGUGAAGAGCUGGCGUUCGCG CCUUCUGGAAACAAAGCGCCCUAGAAACUUGCCUUCACGAGAGACUGGGCUUACCGUGACCGCGGAUGUGGAAGUUCUGAGGGCCUAGAG ACCGUGGUGCAUGACGAAACUUGGCGCuga	603	200
PAC ^{Y171H}	AUGACCGAGUACAAAGCUACAGUGCGGUGGCGACAGAGAUAGUGCCUAGAGCGGUCAGAAACACUGGCGCGUGCCUUCGCGGAUUAUCCUGC CACAAGACACACCGUGGAUCCGACAGACACAUCGAGAGAGUGACCGAGCUGCAAGAGCUUUCUGACAGAGUGCGGCGUGGACAUUGCGGAAAG UGUGGGUUGCAGAUAGUGCGCGCGUGGCGUGUGGACAAACCCUGAAUUCUGGGAAGCGCGCGUGUGUUCGCGGAGUCCGAGCUAGAAUG GCGGAGCUGUGCGGUCUAGAGUGGCGUGCAGCAGCAGAUUGGAAGGACUGUGGCGCCUACAGACCUAAGUGCGAGCGCGCGUGUUCUGC UACCGUGGGAGUGUCCCGUACCAACAGGCAAGGACUGGGAUUCUGUGGUGUGCGGCGUGAAGCGCGUGAAGAGCGUGGCGUUCGCG CCUUCUGGAAACAAAGCGCCCUAGAAACUUGCCUUCACGAGAGACUGGGCUUACCGUGACCGCGGAUGUGGAAGUUCUGAGGGCCUAGAG ACCGUGGUGCAUGACGAAACUUGGCGCuga	603	200
PAC ^N -Int ^N	AUGACCGAGUACAAAGCUACAGUGCGGUGGCGACAGAGAUAGUGCCUAGAGCGGUCAGAAACACUGGCGCGUGCCUUCGCGGAUUAUCCUGC CACAAGACACACCGUGGAUCCGACAGACACAUCGAGAGAGUGACCGAGCUGCAAGAGCUUUCUGACAGAGUGCGGCGUGGACAUUGCGGAAAG UGUGGGUUGCAGAUAGUGCGCGCGUGGCGUGUGGACAAACCCUGAAUUCUGGGAAGCGCGCGUGUGUUCGCGGAGUCCGAGCUAGAAUG GCGGAGCUGUGCGGUCUAGAGUGGCGUGCAGCAGCAGAUUGGAAGGACUGUGGCGCCUACAGACCUAAGUGCGAGCGCGCGUGUUCUGC CUGACCGGGAUUAACGGCCUGCGUCCUAGCGCAAGGACUGGGAUUCUGUGGUGUGCGGCGUGAAGCGCGUGAAGAGCGUGGCGUUCGCG CCUUCUGGAAACAAAGCGCCCUAGAAACUUGCCUUCACGAGAGACUGGGCUUACCGUGACCGCGGAUGUGGAAGUUCUGAGGGCCUAGAG ACCGUGGUGCAUGACGAAACUUGGCGCuga	666	221
Int ^C -PAC ^C	AUGAUCAGAUCGCCACAGGAAGUACUGGGCAAGCAGAACGUGUACGACAUUGCGGUGGAAACGGGACCAACAUCUGCCUGAAGAACCGCUU UAUCCGCGAGCAACUGCGGAGCCGCGUGGUGUUCUUGCUAUCGUGGGAGUGUCCCGUACCAACAGGCAAGGACUGGGAUUCGUGUGGUGCGC CUGGCGUGAAGCGCGUGAAAGAGCUGGCGUUCGCGCUUCUGGAAACAAAGCGCCCUAGAAACUGCCUUCUACGAGAGACUGGCGUACCC GUGACCGCGGAGUGGAAGUUCUGAGGGCCCUAGAAACUGGUGCAUGACAGAAACUGGCGCGCuga	354	117
Int ^C -PAC ^{Cm}	AUGAUCAGAUCGCCACAGGAAGUACUGGGCAAGCAGAACGUGUACGACAUUGCGGUGGAAACGGGACCAACAUCUGCCUGAAGAACCGCUU UAUCCGCGAGCAACUGCGGAGCCGCGUGGUGUUCUUGCUAUCGUGGGAGUGUCCCGUACCAACAGGCAAGGACUGGGAUUCGUGUGGUGCGC CUGGCGUGAAGCGCGUGAAAGAGCUGGCGUUCGCGCUUCUGGAAACAAAGCGCCCUAGAAACUGCCUUCACGAGAGACUGGCGUACCC GUGACCGCGGAGUGGAAGUUCUGAGGGCCCUAGAAACUGGUGCAUGACAGAAACUGGCGCGCuga	354	117

HPH	AUGAAGAAGCCUGAGCUGACCGCUACCAGCGUGGAAAAGUUCUGAUCGAGAAGUUCGACAGCGUGUCCGACCUGAUGCAGCUGUCUGAGGGCGA AGAGAGCAGAGCCUUCAGCUCGAGUUGGCGGCAGAGGCUACGUGCUGAGAGUAAUUCUUGCGCCGACGGCUUCUACAAGGACAGAUACGUGU ACCGGCACUUCGCCUUCUGCUGCCUUCUUCUGAGGUGCUGGACAUCCGGCAGUUCAGCGAGAGCCUAGCCUACUGCAUUUCUGCGAGAGCC CAGGGCGUGAGCCUGAGGAAUUCUUGAACAAGAACUGCCCGCGUGCUGCAGCUGUGGCGUGAAGCUAUGGAUGCCAUUGCCCGCGUGAUCU GAGCCAGACAUUGGCUUUGGCCCUUCGAGCUCUAAAGGCAUCGGCCAGUACACACCCUGGCGGGAUUUUAUCUGGCUAUCCGCGAUCCUCACG UGUACACUCGGCAGACCGUGAUGGACGAUACCGUGUGCUGCCUUCUGGCGCCAGGCUUGGGAACUGAUGCUGUGGCGCGAAGAUUGCCCGAA GUGCGGCAUCUGGUGCACGCGGAUUCUGGCGCAGCAACAACGUGCUGACCGCAACCGCGAGAAUACCCGCGUGAUCGAUUGGAGCGAGGCCAUGUU UGCGGACAGCCAGUACGAGGUGGCGCAUAUUCUUCUGGCGGCCUUGGCGGCGUGCAUGGAACAGCAGACCAGAUACUUCGAGCGGAGACACC CUGAGCUGGCGCGGAUUCUUCUAGACUGAGAGCCUACUUGCUGCGGAUCGCGCUGGAUACAGCUGUACAGUCUCUGGUGGACGCAACUUCGACGAU GCCGCUUGGGCUCAGGGAAGUGAUGCUAUGUGCGGAGCGGAGCGGACAGUGGGGAAGAACACAAUUCGCUAGGAAGAUUCGCCCGUGUG GACCGAUGGCGUGUGGAAGUUCUGGCGCACAGCGGCAUAGACGGCCUAGCACAGACCCAGAGCCAAAGAGuga	1026	341
HPH ^M -Int ^M	AUGAAGAAGCCUGAGCUGACCGCUACCAGCGUGGAAAAGUUCUGAUCGAGAAGUUCGACAGCGUGUCCGACCUGAUGCAGCUGUCUGAGGGCGA AGAGAGCAGAGCCUUCAGCUCGAGUUGGCGGCAGAGGCUACGUGCUGAGAGUAAUUCUUGCGCCGACGGCUUCUACAAGGACAGAUACGUGU ACCGGCACUUCGCCUUCUGCUGCCUUCUUCUGAGGUGCUGGACAUCCGGCAGUUCAGCGAGAGCCUAGCCUACUGCCUGAGCUCACGAGACA GAGAUCCUGAGCCGGAUUAACGGCCUGCUGCCUACCGCAAGAUUGGGAAGCGGAUCGAGUGCACCGUGUACUCCGUGGAUACCGCGCAAC CAUCUACACCCAGCCUGUGGCUAGUGGCACGACAGAGGCGAGCAAGAGGUGUUCGAGUACUGCCUGGAAGAUUGGCGAGCCUGAUCAGAGCCACA AGGACCAAGUUCUAGACAGUGGACGGCCAGAUUGCGCCUACGACGAGAUUCUUGAGCGGAGCGUGGACCUGAUGAGAGUGGACAACCGCCU AACuga	576	191
Int ^C -HPH ^C	AUGAUAAGAUCGCCACACGGAAGUACUGGGCAAGCAGAACGUGUACGACAUCGGCGUGGAACGGGACCACAACUUCGCCUGAAGAACCGCUU UAUCGCCAGCAACUGCAUUUCUGGAGAGCCAGGGCGUGACCCUGCAGGAUUCUUCUGAACAAGACUUGCCGCGUGCUGCAGCCUGUGGCGU AAGCUAUGGAUUGCCAUUGCCGCGUGAUCUGAGCCAGACAUCUGGCUUUGGCCUUCUGGACCUAAGGCAUCGGCCAGUACACCCACUGGCGG GAUUUAUCUGGCUAUCGCGGAUCCUACGUGUACCAUCUGGCGAGCCUGAUGGACGAUACCGUGUGCCUUCUGUGGCGCCAGGCUUCGGAUGA ACUGAUCUGUGGCGCGAAGAUUGCCCGAAGUGCGGCAUCUGGUGCACGGCCGAUUUCGGCAGCAACAACGUGCUGACCGCAACCGCAGAAUCA CCGCGGUGAUCGAUUGGAGCGAGGCCAUGUUGGCGCAGCCAGUACGAGGUGGCGCAUAUUCUUCUGGCGGCCUUGGCGUGGCGUGCAUGGAA CAGCAGACCAAGAUUCGAGCGGAGACACCCUGAGCUGGCGGAUUCUUCUAGACUGAGGCUACUAGCUGCGGAUCGGCCUGGGAUCAGCUGUA CCAGUUCUGGUGGACGGCAACUUCGACGAUGCCGCUUGGCGCUCAGGGAAGAUUGAUGCUAUGUGCGGAGCGGAGCCGGCACAGUGGGAAGAA CACAAUUCGCUAGAAGAUUCUGCCCGCGUGUGGACCGAUGGCGUGUGGAAGUUCUGGCGCACAGCGGCAUAGACGGCCUAGCAACAAGCCAGA GCCAAAGAGuga	867	288
BSR	AUGAAGACCUUCAACAUCAGCCAGCAGGACUGGAAACUGGUGGAAGUGGCCACCGAGAAGAUACCAUCGUGUACGAGGACAAACAGCACCAGU GGGAGCCGCCAUCAAGAACAAAGACCGCGGAGAUCAUCAGCGCCGUGCACAUUCGAGGCCUACAUUGGCGAGAGUAGAGUGGCGCCGAGGCCAUUG CUAUCGGAAGCGCCUGUCUAACGGCCAGAGGACUUCGAUACCAUCUGGCGCCGUGCGGCACCCUACAGCGACGAAGUGGACAGAUCCAUCA GUGGUGUCCCAUCGCGCAUGUGCAGAGAGCUGAUCUUCGACGCCCCUGACUGCUCUGUGCUGAUCGAGAUAGAACGGCAAGCUGGUAAGAG CACCAUCGAGGAACUGAUCCUUCUGAAGUACACCCGGAACuga	423	140
BSR ^M -Int ^M	AUGAAGACCUUCAACAUCAGCCAGCAGGACUGGAAACUGGUGGAAGUGGCCACCGAGAAGAUACCAUCGUGUACGAGGACAAACAGCACCAGU GGGAGCCGCCAUCAAGAACAAAGACCGCGGAGAUCAUCAGCGCCGUGCACAUUCGAGGCCUACAUUGGCGAGAGUAGAGUGGCGCCGAGGCCAUUG CUAUCGGAAGCGCCUGUCUAACGGCCAGAGGACUUCGAUACCAUCUGGCGCCGUGCGGCACCCUACAGCGACGAAGUGGACAGAUCCAUCA GUGGUGUCCCAUCGCGCAUGUGCCUGAGCUACGAGACAGAGAUCCUGACCGUGGAAUACGGCCUGUGCUUAUCGGCAAGAUUGGGAAGAGG GAUCGAGUGCACCGUGUAUCUGGGAUAACACGCAACAUUACACCCAGCCUGUGGCUAGUGGCAAGACAGGCGGAGCAAGAGGUGUUCG AGUACUGCCUGGAAGAUUGGCGCCUGAUCAGAGCCACCAAGGACCAAGUUAUGACAGUGGACGGCCAGAUUGCUGCCAUUCGAGAGAUUCU GAGCGGAGCUGGACCGUAUGAGAGUGGACAACCGUCCUAACuga	615	204
Int ^C -BSR ^C	AUGAUAAGAUCGCCACACGGAAGUACUGGGCAAGCAGAACGUGUACGACAUCGGCGUGGAACGGGACCACAACUUCGCCUGAAGAACCGCUU UAUCGCCAGCAACUGCAGAGAGCUGAUUCGACUACGCCCCUGACUGCUCUGAUCGAGUAAGCGGCAAGCUGGUAAGGACCAACUUC AGGAACUGAUCCUUCUGAAGUACACCCGGAACuga	225	74
TKA _{266C}	AUGGCCAGCUAUCCUUGUACACGACAGCCAGCGCCUUGAUCAGGCGCAAGAUUCAGAGGCCACAGCAACAGAAAGACAGCCUUGCGGCCUUG GAGACAGCAAGAGGCUACAGAAUUCGGCUGGAACAGAAUGCCACACUUGCUGCGGGUGUACAUUGGCGCCUACGCGCAUGGGCAAGACCA CCACAACACAGCUGCUGGUGGCCUUGGCGAGCAGAGAUUAUCUGUGUACUGGCGCCGAGCUCUAGACCUAUGGCGAGGUGUUGGAGCCAGCGAG ACAUCGCCAACAUUACACACACAGCAGCCGCGUGAUCAGGCGCAAAUUCUGUGGCGAGCGCCGUGGUAUGAUAUCUUGCCAGAUAC CAUUGGGAUUGCUUACGCCUGACAGAUUGCUGUGGCCUUCACAUUGGCGGAAGAACGGGAUUCUUCUAGUCCCCACUCCAGCUCUGACCA UCUUCUGGACAGACACCCUACGCCUUCUAGCUGUGUUAACCGCGCCAGAUACCUAUGGCGCAGAUACACCUAGGCCGUGUGGCUUUC GUGGCCUGAUUCCUUCACUUGCCCGCACCAUUCGUGUGGAGGCCUUGCUGAGGACCGGCACAUUGAUAGACUGGCCAAAGAGACAGCG GCCUGGCGAGAGAUUGGUAUCUGGCGGCCUACAGAAAGUGUACGGCCUGUGGCCAACACCCUGCGGUUUCUUAAGCGCGCGGAU CUUGGAGAGAGGACUGGGACAUCUAGCGGCCACAUGUUCUCCACAAGGCGCCGAGCCUAGUUAUUGCUGGACCCAGACCUACAUUGCGG GACACCCUGUUAUCCUGUUCAGAGCCCGUGAGCUGGCGUUCUUAACGGCGACCUUGUACCAACGUGUUGCCUGGCGCUUUGACGUGCUGGCAAA AAGACUGCGGCGCAUGCACGUGUUAUCUUGGACUACGAUACUCCCGCGCGUGAAGAGUUCUUGCUGCAGCUGACAGCAAGCGCAUGGUGC AGACCCAGUUAACAACCCUGGCGACAUCCCCACCAUCUGUGACUUGGCCAGAACUUCGCCAGAGAGUUGGGGAAGCCAAACuga	1131	376

NLS-spCas9 ^N - Int ^N	AUGCCCAAGAAGAGAGGAAGGUGAUGGACAAGAAAGUACUCCAUGGGGCUCAUAUCGGCACAAACAGCGUGGGCCGUCUUAUACGGACGA GUACAAGGUGCGGAGCAAAAAAUUCAAAGUUCUGGGCAUACCGAUCCGACAGCAUAAAGAAAGAACCCUUAUUGGCGCCUCCUGUUCGACUCCG GGGAGACGGCCGAAGCCACGGGCUAAAAAGAACAGCACGGCGCAGAUUAACCCGAGAAAGAAUCGGAUUCGUUACCUAGCAGGAGAUUUUAGU AAUGAGAUUGCUAAGGUGGAGUACUUCUUCUUAAGGCGUGGAGGAGUCCUUUUGGUGGAGGAGGAUAAAAAGCACGAGCGCCACCCAAUCU UGGCAUAUCUGGAGCAGGUGGCGUACCAUGAAAAGUACCCAAACCAUAUAUCAUCUGAGGAAGAAAGCUUGUAGACAGUACUGAUAAGGCGUACU UGCGGUUGAUCAUCUCGCGUGGCGCAUAUGAUCAAAUUCGGGGACACUCCUUAUCAGGAGGGGACCUAGAACCCAGACAACAGCGAUUCGAC AAACUUCUUUAUCCAAUCUGGUUACAGAUUACAACAGCUUUUCGAAAGAAACCCGAUCAACGCAUCCGGAGUUGACGCCAAAGCAAUCCUGAGCGC UAGGCGUUCCAAUCCCGCGGCUCAAAACCUCAUCGCAAGCUCUCCUGGGGAGAAAGAAACGGCCUGUUUGGUAUUAUUCGCCCUGUAC UCGGGCGUACCCCCAACUUUAAAUCAUACUUGACUCCUGGCGGAAGAUCCAGCUCUACUAGAGCAAAGACACCUACGAUGAUGAUUCGACAAU CUGCUGGGCCAGAUCCGGACAGUACGCGAGACCUUUUUUGGGCGCAAGAACCUUACAGACGCCAUUCUGCUGAGUGAUUUUCGCGAGUGAA CACGGAGAUCAACAAAGCUCUCCUGAGCGCUAGUAUGAUCAAGCGCUAUGAUGAGCACCAAGACUUGACUUGCUUAGAGGCCUUCUGACAG AGCAACUGCCUGAGAAAGUACAAGGAAAUUUUCUUCGAUCAGUCUAAAAUUGGCUACGCGGGAUACAUAUGACGGCGAGCAAGCCAGGAGGAUUU UACAAAUUUUAAGCCCAUCUUGGAAAUUAGGACGGCACCGAGGAGCUGCUGGUAAGCUUAAACAGAGAAAGAUUCUGUUGCGCAACAGCGCAC UUUCGACAAGGAAGCAUCCCCACCAAGAUUACCCUGGGCGAACUGCACGCUAUCUACGGCGGCAAGAGGAUUUUAACCCUUUUUUGAAAGAU ACAGGGAAGAAUUGAGAAAUUCCUACAUUUCGGAUACCCUACUAUGUAGGCCCCUUGCCCCGGGAAAUUCCAGAUUCGCGUGGAUAGACUCCG AAAUACAGAAAGACCAUACUCCUGGAACUUCGAGGAAGCUGGGAUAAAGGGGCGCUCUGCCAGUCCUUAUCGAAAGGAUACUUAUUCGUA UAAAAAUUCGCUAAACGAAAGGUGCUUUAACACACUCUCUGCUGUACGAGUACUACAGUUUAUAAAGAGCUCACCAAGGUCAAAUAGCUC CAGAAAGGAUGAGAAAGCCAGCAUUCUUGCUGGAGAGCAGAAAGAUUACUGGAGACCCUUCUUAAGACGAAACCGGAAGAUUCCGUGAA CAGCUCAAAGAAAGCAUUAUUAACAAAGAUUGAAUUGCCUGCUACGAGACAGAGAUCCUGACCCUGGGAUACGGCCUGGCUUCCUGGCUA CGUGGAAAGCGGAUCGAGUGCACCUGUACUCCUGGGAUAAACAGGGCAACAUACACCCAGCCUGUGGCUAGUGGCGACGACAGAGGCGAGC AAGAGGUGUCCAGUACUGCUGGAAAGUGGACUGAUCAGAGCCACCAAGGACCAAGAUUCAUGACAGUGGACGGCCAGAUUCGCCCCAUC GACGAGAUUCUGAGCGGAGCUGGACCUGAUGAGAGUGGACAACUUGCCUACUGa	2052	683
Int ^C -spCas9 ^C - NLS	AUGAUCAAGAUCGCCACACGGAAGUACUUGGGCAAGCAGAGACGUGUACGACAUCCGGGUGGAACGGGACCAACUUCGCCUUGAAGAACGGCUU UAUCCGCCAGCAACUGUUUCGACUCUGUUGAAUACGCGGAGUGGAGGAUCGUCUUAACGCAUCCUGGGAAACGUUAUCACGAUUCUCCUGAAAUCA UUAAAGACAAGGACUUCUGGCAAAUAGGAGAGACGAGGACAUCUUCUGAGGACAUCUUCUCCACCCUACCGUUGUUUGAAGAUAGGAGAGAUUAU GAAGAAGCGCUUGAAACUUACGCUCAUCUCUUCGACGACAAAGUCAUGAAACAGCUCUAGAGGGCCCGAUUAACAGGAUUGGGGCGGCUUGCAAG AAAAUCGAUCAUAGGGAUCCGAGACAAGCAGAGUGGAAGACAUAUCCUGGAUUUUUUAAGUCCGAGUGGAUUUGCAACCGGAACUUAUGCAGU UGAUCCAUAGAUAGCUCUCACCUUUAAAGGAGGACAUCGAGAAAGCACAAGUUUCUGGCCAGGGGACAGUCUUCACGAGCACAUCCGUAUUUU GCAGGUAGCCCGAUCAAAAGGGAUUCUGCAGACCGUUAAAGGUCUGGGAUAAUCUGCUCAAGAAUAAUGGGAAGGCAUAAAGCCGAGAAUUAU CGUUUAUCGAGAUUGGCCGAGAGAACCAACUACCCAGAGGGACAGAGAAACAGUAGGGAAGGAUUAAGAGGGAUUAAGAGGGUAUAAAGAAAC UGGGUCCCCAAUUCUUAAGAAACACCCAGUUGAAACACCCAGCUUCAGAAUGAAGACUUAUCCUGUAUACUUGCAGAAACGGCAGGGAUUG UACGUGGAUCAGGAACUGGACAUAUCCGGCUCUCCGACUACGACGUGGAUCAUUCUGUGCCCCAGUCUUUUUCAAGAAUUAUUAUUGUAUA UAAAGUUGUACAGAAUCCGAUAAAAUAGAGGGAAGAGUUAACGUCCCUCAAGAAAGAUUGUCAAGAAAUUAGAAAAUUUUGGCGGCGAGC UGCUUAACGCGCAACUGAUCAACACAGGAAGUUCGAUAAUUGUACUUAAGGCUGAACAGGUGGCGCUGUCUGAGUUGGAUAAAGCCGGCUCUAC AAAAGGCAGCUGUUGAGACAGCCAGAUCAACCAAGCAGGUGGCCAAAUUCUGAUUUCACGCAUGAACACCAAGUACGAUAAAAUAGCAAAU GAUUCGAGAGGUGAAAGUUUAUUCUGAAGUCUAAGCUGGUCUAGAUUUCAGAAAGGACUUCAGUUUAUAAAGGUGAGAGAUCAACAAU ACCACCAUGCGCAUGAUGCCUACCUAGAUAGCAGUGGUAGGCAUCGACUUAUCAAUAAAUUCCCAAGCUUGAAUUGUUUAUCGAGAC AUUAAAGUGUACGAUGUUAAGAAAUUAUGCAGAAAGUCUGAGCAGGAUUAAGGCAAGGCCACCGCUAAGUAUUCUUUAACAGCAAUUAUUAUG UUUUUUAACAGCCGAGAUUAACUGCGCAAGUAGGAGAGAUUCGAAAGCGACCAUUAUUGCAAAACAAAGGAAACAGGAGAAUUCGUGUGGACA AGGGUAGGGAUUUCGCGACAGUCCGGAAGGUCUUCUUAUGCGCAGGUGAACAUUGUUAAAAAGACCGAAGUACAGACCGGAGGCUUCUCCAG GAAAGUAUCCUCCGAAAGGAACAGCGACAAGCUGAUCGACGCAAAAGAAUUGGAGCCCAAGAAAUACGGCGGAUUCGAUUCUCCUACAGU CGCUUACAGUGUAUCUGGUUGGCGCAAGUGGAGAAAGGGAAGUCUAAAAAACUCUAAAGCGCUAAGGAACUGUGGGCAUACAUUACUGGAGC GAUCAAGCUUCGAAAAAACCCCAUCGACUUCUUCGAGGCGAAAGGAUUAUAAAGAGGUCAAAAGAGACCUCAUUAUAAAGCUCCAGUACUCU UCUUCUUGAGUCUUAAGAAACGGCCGGAACGAAUUGCUCUAGUGCGGGCGAGCUGCAGAAAGGUAAACGAGCUGGCAUCUGCCCUUAAAUACGUUAA UUUCUUGAUUCUGGCCAGCCAUUGAAGAAAGCUAAAGGGUCUCCCGAAGAUUAUGAGCAAGAGCAGCUGUUGGGAACAAACAAACACUACCC UUGAUGAGAUCAUCGAGCAAAUAAAGCAUUCUCCAAAGAGUGAUUCUUGCGGACGCUUAACUUGAUAAGGUGCUUUUGCUUAACAAUAAAGCAC AGGGAUAAAGCCUACAGGAGAGCAGGAGAAACAUUAUCCACUUGUUUACUCUGACCAACUUGGCGCGCCUGCAGCCUUAAGUACUUCGACAC CACCAUAGACAGAAAGCGGUACACCUUACAAAGGAGGUCUGGACGCCACAGUUAUCAGUCAAUUAACGGGCUUAUGAAGAAACAGAAUUCG ACCUUCUCAGCUGGUGGAGACAGCAGGCGUGACCCAGAGAGAGAGAGGUGa	2529	842
L7Ae	AUGUACUGAGAUUUUGAGGUUCUGGAGCAUCAGCAACGAAGCUCUGAUUCUGUGGAGAAAGGUUAGGAGAGCGGUUAGGUAAGAAAGGUAUC CAACGAGACGACAAAGGCUUGGAGAGGGGACUGGCAAGAGCUCGUUUACAUCGACAGAGGAUUGUAGCCGCCUGAGAUUCGUUCUACUUGCCCC UCCUCUGCGAGGAGAAAGAUUGGCGUACAUUUACGUUAAAGCAAGAACGACCUUGGAAGGGCUUGGGCAUUGAGGUGCCAUUGCGCUUCGGCA CGCAUAUACAAAGGAGAGCUGAGAAAGGAGCUUGGAAGCCUUGUGGAGAAAGUAUAAAGCCUUCAGAGUGa	360	119
LIN28A	AUGGGCAGCGUGUCCAAUCAGCAAUUUGCGCGGGAUGUGCCAAGGCCGCUAGAAGAGCUCAGAAAGACCCUGAGGAGCGCCGACAGAGCUGC UGAUGAACCUCAACUGCUUCACGGCGCUGGCAUCUGCAAGUGGUUCAAUGUGCGGAUGGGCUUCGGCUUCUGAGCAUGACAGCUAGAGCGUGCG UGGCCUUGGAUCCUUGUGGAUGUUGUUGGACACAGAGCAAGCUGCACAUGGAAAGGCUUCGCCAGCCUGAAAGAGGGCGAAGCCUUGAGUUC ACCUUUAAGAAUGUCCAAAGGCCUCGAGAGCAUCAGAGUACAGGACCUUGCGGAGUGUUCUGCAUCUGGCUUCUGAGAGAAAGGCCAAAGGGCAA GAGCAUGCAGAAAGCGGAGAGCAAGGGCGACAGAUUCUACUUGUGGCGGCCUGGAUACCAACGCCAAAGAGUGCAACUUGCCUUCUACGCCUA AGAAGUGCCAUUUUGCCAGAGCAUCCCAUAGUGGCGCAGCUGUCCUUGAAAGCUCAGCAGGAGGACUAGCGCUCAGGGCAAGGCCUACCUAC UUCAGAGAGGAAGGAGAAAUUACAGCCCCACACUGUGCCGAGGCUAGAAUUGa	630	209

AG ^{v1535} -bN	AUGGUGAGCGUGAUCAAAGCCGAGAUAGAUAAGCUGUGCAUGAGGGGCACCGUGAACGGCCACAACUUCUGUAGCGGGCGAGGGCAAAGGG CAACCCUACGAGGGCACCCAGAUCCUGGACCCUGAACGUGACCGAGGGGCCCCCUGCCUUCGCCUACGACAUCCUGACCACCGUUGUCUGCU ACGGCAACAGGGCCUUCACCAAGUACCCCGCCGACAUCCAGGACUACUUCACAGCAGACCUUCCCGAGGGCUACCAUGGGAGAGGAGCAUGACC UACGAGGACCAAGGGCAUCUGACCCGCCACCAGCAACAUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUCGACGGCCACAACUUC CCCCAACGGCCCGUGUAGCAGAAGAAGACCCUGAACUGGGAGCCAGCACCGAGAAGAUGUACGUGGAGGACGGCUGCAUCAGCGCGCAUAGCC UGAUUCUUCUGGCCAGCACAGGCAAGAGAGUGCCCAUCAAAGGAUCUGUGGGCGAGAGGACUUCGAGAUUCUGGGCAUCAAACGAGCAGACCAUG AAGCUGGAAAGCGCCAGGUGUCCCGGGUUCUGCAACCGGAAGAACUGGUGUACACACUGAAACCCCGGCGGAGCAAGCAUCAAAGGCCAC CGCCAAACCCGGUUCUGACAAUCGACGGCUGGAAGAGACUGGACGAGCUGAGCCUGAAAGAGCACAUUGCCUGCCUAGAAAGCUGGAUCCA GCGCCUGCAGuga	774	257
AG ^{v1535} -bC	AUGCUGGCCCCUGAGAUUGAGAAGCUGCCCGAGAGGACAUACUUGGGACCCCAUCUGUCCAUCACCGAGACAGGCGUGGAAGAGGUGUUCGA CCUGACAGUGCCCGCCUGAGAAACUUCUGGGCCAAACGACAUCAUCUGGCAACAACGUGAAGGGCGACGUGAACAUAGGCGUGCUGUGGAGG GCGGCGGCCACUACAGGUGCGACUUAAGACCACCUCAAGGCCAAGAAGGAGGUGAGGCGCCGACGCCACAAGAUUCGACACAGGAUCGAG AUCCUGAAGCACGACAAGGACUACAACAAGGUAAGCUGUACGAGAACCGGUGGCCAGGUACUCCAUGCUGCCAGCCAGGCCAAGuga	375	124
AG ⁶¹⁶⁷⁵ -aN	AUGGUGAGCGUGAUCAAAGCCGAGAUAGAUAAGCUGUGCAUGAGGGGCACCGUGAACGGCCACAACUUCUGUAGCGGGCGAGGGCAAAGGG CAACCCUACGAGGGCACCCAGAUCCUGGACCCUGAACGUGACCGAGGGGCCCCCUGCCUUCGCCUACGACAUCCUGACCACCGUUGUCUGCU ACGGCAACAGGGCCUUCACCAAGUACCCCGCCGACAUCCAGGAUACUUCACAGCAGACCUUCCCGAGGGCUACCAUGGGAGAGGAGCAUGACC UACGAGGACCAAGGGCAUCUGCACCCGCCACCAGCAACAUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUCGACGGCCACAACUUC CCCCAACGGCCCGUGUAGCAGAAGAAGACCCUGAACUGGGAGGCCAGCACCGAGAAGAUGUACGUGAGGACGGCGUGUGAAGGGCGAGGUGA ACAUGAGGCGUGCUGGAGGGCGUGCAUCAGCGCGCAUAGCCUGAUUCUUCUGGCCuga	534	177
AG ⁶¹⁶⁷⁵ -aC	AUGAGCAGGGCAAGAGAGUGCCCAUCAAGGAUCUGUGGGCGAGAAGGACUUCGAGAUUCGGGCCAUCAACGAGCAGACCAUGAAGCUGGAAAG CGCCAAGGUGUCCCGGGUGUUCUGCACCGGAAAGAAACUGGUGUACACACUGAAACCCGGCGUGGCGAGAACCAUAAGGCCACCGCCAAACACC GGUUCUGACAAUACGAGCGGUGAAGAGACUGGACGAGCUGAGGCGUGAAGAGCACAUCUGCCUGCCUGAAGACUGGAAUCCAGCAGCCUGCAG CUGGCCCCUGAGAUUGAGAAGCUGCCCGAGGCGACAUUCUGGGACCCCAUCUGGUGUCCAUCACCGAGACAGGCGUGGAAGAGGUGUUCGACG GACAGUGCCCGCCUGAGAAACUUCUGGGCCAAACGACAUCAUCUGGACACAACGCGCCACTACAGGTGCGACTTCAAGACCACCTACAAGGCCA AGAAGGAGGTGAGGCTGCCCGACGCCACAAAGATCGACCAAGGATCGAGATCCTGAAGCAGCACAAGGACTACAACAAGGTGAAGCTGTACGAG AAGCCGTGGCCAGGTACTCCTGCTGCCAGCCAGGCCAAGtga	615	204
AG ⁶¹⁶⁷⁵ -bN	AUGGUGAGCGUGAUCAAAGCCGAGAUAGAUAAGCUGUGCAUGAGGGGCACCGUGAACGGCCACAACUUCUGUAGCGGGCGAGGGCAAAGGG CAACCCUACGAGGGCACCCAGAUCCUGGACCCUGAACGUGACCGAGGGGCCCCCUGCCUUCGCCUACGACAUCCUGACCACCGUUGUCUGCU ACGGCAACAGGGCCUUCACCAAGUACCCCGCCGACAUCCAGGACUACUUCACAGCAGACCUUCCCGAGGGCUACCAUGGGAGAGGAGCAUGACC UACGAGGACCAAGGGCAUCUGCACCCGCCACCAGCAACAUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUCGACGGCCACAACUUC CCCCAACGGCCCGUGUAGCAGAAGAAGACCCUGAACUGGGAGGCCAGCACCGAGAAGAUGUACGUGGAGGACGGCGUGUGAAGGGCGAGGUGA ACAUAGGGCUGUGCUGGAGGGCGUGCAUCACGCGCGAUACGUGCUGAUUCUUCUGGCCAGCACAGGCAAGAGAGUCCCAUCAAGGAUUCUGCGGGC GAGAAGGACUUCGAGAUUCGGGCCAUCAACGAGCAGACCAUGAAGCUGGAAAGGCCCAAGGUGUCCCGGGUGUUCUGCACCCGAAAGAAACUGGU GUACACACUGAAACCCCGGCGUGGCGAGAACCAUCAAGGCCACCGCCACACCCGGUUCUGACAAUCGACGGCUGGAAGAGACUGGACGAGCUGA GCCUGAAGAGCACAUUGCCUGCCUAGAAAGCUGGAUCCAGACGCCUGCAGuga	816	271
AG ⁶¹⁶⁷⁵ -bC	AUGCUGGCCCCUGAGAUUGAGAAGCUGCCCGAGAGCGACAUCAUCUGGGACCCCAUCUGUCCAUCACCGAGACAGGCGUGGAAGAGGUGUUCGA CCUGACAGUGGCCCGCCUGAGAAACUUCUGGGCCAAACGACAUCAUCUGGCAACAACGCGCCACTACAGGTGCGACTTCAAGACCACCTACAAGG CCAAGAGGAGGTGAGGCTGCCCGACGCCACAAGATCGACCAAGGATCGAGATCCTGAAGCAGCACAAGGACTACAACAAGGTGAAGCTGTAC GAGAACCGCTGGCCAGGTACTCCTGCTGCCAGCCAGGCCAAGtga	333	110
Npu ^C -AG ^{mid} - Ssp ^N	AUGAUCAAGAUCGCCACACGGAAGUACUGGGCAAGCAGAAAGUGUACGACAUUCGGCGUGGAACGGGACCAACAUCUGCCUGAAGAACGGCUU UAUCGCCAGCAACUGCUACGGCAACAGGGCCUUCACCAAGUACCCCGCCGACAUCCAGGACUACUUAAGCAGACCUUCCCGAGGGCGUACCAU GGGAGAGGAGCAUAGCCUACAGGACAGGGCAUCUGCACCCGCCACAGCAACAUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUC GACGGCACCAACUUCUCCCGCCAAACGGCUGCAUCAGCGGCGAUAGCCUGAUUCUUCUGGCCAGCACAGGCAAGAGAGUGCCCAUCAAGGAUCUGCU GGGCGAGAAGGACUUCGAGAUUCGGGCCAUCAACGAGCAGACCAUGAAGCUGGAAAGCGCCAAAGGUGUCCCGGGUGUUCUGCACCGGAAAGAAAC UUGUGUACACACUGAAACCCCGCGUGGCGAGAACCAUAGGCCACCGCCAAACCGGUGUUCUGACAAUCGACGGCUGGAAGAGACUGGACGAG CUGAGCCUGAAGAGACAUUCGCCUGCCUAGAAAGCUGGAUCCAGCAGCCUGCAGuga	630	209
AG ^{mid} m-Npu ^N	AUGUGCUACGGCAACAGGGCCUUCACCAAGUACCCCGCCGACAUCCAGGACUACUUAAGCAGACCUUCCCGAGGGCGUACCAUGGGAGAGGAG CAUGACCUACGAGGACAGGGCAUCUGCACCCGCCACCAGCAACAUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUCGACGGCACCA ACUUCUCCCGCCAAACGGCUGCCUGAGCUACGAGACAGAGAUCUGACCGUGGAUAACGGCCUGCUGCCUACUGGCAAGAUUCUGGAAAGCGGAU GAGUGCACCGUGUACUCCUGGUAUACAACGGCAACAUCUACACCCAGCCUGUGGCUAGUGGCGACGACAGAGGCGAGCAAGAGGUGUUCGAGUA CUGCCUGGAAGAGGACGCGUGAUACAGGCCACCAAGGACCAAGUUAUGACAGGAGCGGCCAGAUUGCCCAUCGACGAGAUUCUGGAGC GCGAGCUGGACUGAUGAGAGGAGCAACUGCCUACuga	516	171
Npu ^C -AG ^{mid} m- Ssp ^N	AUGAUCAAGAUCGCCACACGGAAGUACUGGGCAAGCAGAAAGUGUACGACAUUCGGCGUGGAACGGGACCAACAUCUGCCUGAAGAACGGCUU UAUCGCCAGCAACUGCUACGGCAACAGGGCCUUCACCAAGUACCCCGCCGACAUCCAGGACUACUUAAGCAGACCUUCCCGAGGGCGUACCAU GGGAGAGGAGCAUAGCCUACAGGACAGGGCAUCUGCACCCGCCACAGCAACAUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUC GACGGCACCAACUUCUCCCGCCAAACGGCUGCAUCAGCGGCGAUAGCCUGAUUCUUCUGGCCAGCACAGGCAAGAGAGUGCCCAUCAAGGAUCUGCU GGGCGAGAAGGACUUCGAGAUUCGGGCCAUCAACGAGCAGACCAUGAAGCUGGAAAGCGCCAAAGGUGUCCCGGGUGUUCUGCACCGGAAAGAAAC UUGUGUACACACUGAAACCCCGCGUGGCGAGAACCAUAGGCCACCGCCAAACCGGUGUUCUGACAAUCGACGGCUGGAAGAGACUGGACGAG CUGAGCCUGAAGAGACAUUGCCUGCCUAGAAAGCUGGAUCCAGCAGCCUGCAGuga	630	209
Ssp ^C -AG ^{mid} m	AUGCUGGCCCCUGAGAUUGAGAAGCUGCCCGAGAGCGACAUCAUCUGGGACCCCAUCUGUCCAUCACCGAGACAGGCGUGGAAGAGGUGUUCGA CCUGACAGUGGCCCGCCUGAGAAACUUCUGGGCCAAACGACAUCAUCUGGCAACAACUGCUACAGGCGCCACTACAGGTGCGACTTCAAGACCACCTACAAGG UCCAGGACUACUUAAGCAGACCUUCCCGAGGGCGUACCAUGGGAGAGGAGCAUAGCCUACGAGGACCAAGGCAUCUGCACCCGCCACCAGCAAC AUCAGCAUGAGGGGCGACUGUCUUCUACGACAUACAGGUUCGACGGCACCAACUUCUCCCGCCAAACGGCUGA	357	118

5'UTR			
5UTR	aggcgaacuaguuuucucuggucccccacagacucagagagaacccgccacc	52	-
Min5UTR	aggagacugccacc	14	-
5UTR-t21	aggcgaacuaguuuucucuggu <u>ucaacaucagucugauaagcu</u> agccacc	52	-
5UTR-t302a5p	aggcgaacuaguuuucucuggu <u>agcaaguacauccacguuu</u> aagugccacc	53	-
5UTR-t206	aggcgaacuaguuuucucuggu <u>ccacacacuuccuacauucca</u> gccacc	52	-
3'UTR			
mtRNR1-AES 3UTR	ugaggauccgaucugguacugcaugcacgcgaauagcuagcugcccccuuucccguccuggguaaccccgagucuccccgaccucggguccagguau gcuccccaccuccaccugcccccacacaccucugcuaguuuccagacaccucccaagcacgcagcaaugcagcucaaaacgcuuagccuagccac acccccacgggaaacagcagugauuaacuuuagcauaaaacgaaaguuuaacuaagcuauacuaaccccgaggguuggucauuucgugccagcc acacc	290	-
Extra sequence			
t21-deaKu5g	<u>ucaacaucagucugauaagcu</u> aaccuocggaaaccguagauuccgggocggucggagccgcccgggagcugaguucucccgcg <u>ccauggguggcuuu</u> uug	98	-
t302a5p-deaKu5g	<u>agcaaguacauccacguuu</u> aaguaccuocggaaaccguagauuccgggocggucggagccgcccgggagcugaguucucccgcg <u>ccauggguggcuu</u> uuug	99	-
t206-deaKu5g	<u>ccacacacuuccuacauucca</u> aaccuocggaaaccguagauuccgggocggucggagccgcccgggagcugaguucucccgcg <u>ccauggguggcuuu</u> uug	98	-
gRNA			
EGFP-targeting	<u>GGGCACGGGCAGCUUGCCGG</u> guuuuagagcuagaaaagcaaguuuuuuuuuagggcuaguccguuuuacuuugaaaaaguggcaccgagucggug uuuu	99	-
DMDex45-targeting	<u>GGGUUUCUUACAGGAACUCC</u> guuuuagagcuagaaaagcaaguuuuuuuuuagggcuaguccguuuuacuuugaaaaaguggcaccgagucggug uuuu	99	-

Supplementary Table 2

Description of mRNAs used in this study.

#	Name	Description	CleanCap AG or CleanCap AG 3'ome	5'UTR	ORF	3'UTR	length of poly(A)	extra sequence
S1	21switch_ctrl	CC-Min5UTR-hmAG1-3UTR-A100	CC	Min5UTR	hmAG1	3UTR	100	-
S2	21switch_a	CC-Min5UTR-hmAG1-3UTR-A100-t21-ak	CC	Min5UTR	hmAG1	3UTR	100	t21-ak
S3	21switch_d	CC-Min5UTR-hmAG1-3UTR-A100-t21-de	CC	Min5UTR	hmAG1	3UTR	100	t21-de
S4	21switch_ad	CC-Min5UTR-hmAG1-3UTR-A100-t21-ak-de	CC	Min5UTR	hmAG1	3UTR	100	t21-ak-de
S5	21switch_da	CC-Min5UTR-hmAG1-3UTR-A100-t21-de-ak	CC	Min5UTR	hmAG1	3UTR	100	t21-de-ak
R	iRFP	CCm-5UTR-iRFP670-3UTR-A120	CCm	5UTR	iRFP670	3UTR	120	-
1	AG	CC-5UTR-hmAG1-3UTR-A100	CC	5UTR	hmAG1	3UTR	100	-
2	AG _{Q63C}	CC-5UTR-hmAG1 _{Q63C} -3UTR-A100	CC	5UTR	hmAG1 _{Q63C}	3UTR	100	-
3	AG _{Q63C, G65A}	CC-5UTR-hmAG1 _{Q63C, G65A} -3UTR-A100	CC	5UTR	hmAG1 _{Q63C, G65A}	3UTR	100	-
4	AG ^N -Int ^N	CC-5UTR-AG ^N -Int ^N -3UTR-A100	CC	5UTR	AG ^N -Int ^N	3UTR	100	-
5	Int ^C -AG ^C	CC-5UTR-Int ^C -AG ^C -3UTR-A100	CC	5UTR	Int ^C -AG ^C	3UTR	100	-
6	Int ^C -AG ^C m	CC-5UTR-Int ^C -AG ^C m-3UTR-A100	CC	5UTR	Int ^C -AG ^C m	3UTR	100	-
11	AG _{Q63C} -ON ₂₁	CC-5UTR-hmAG1 _{Q63C} -3UTR-A100-t21-deaKu5g	CC	5UTR	hmAG1 _{Q63C}	3UTR	100	t21-deaKu5g
12	AG ^N -Int ^N -ON ₂₁	CC-5UTR-AG ^N -Int ^N -3UTR-A100-t21-deaKu5g	CC	5UTR	AG ^N -Int ^N	3UTR	100	t21-deaKu5g
12m	AG ^N -Int ^N -ON ₂₁	CCm-5UTR-AG ^N -Int ^N -3UTR-A100-t21-deaKu5g	CCm	5UTR	AG ^N -Int ^N	3UTR	100	t21-deaKu5g
13	Int ^C -AG ^C -ON ₂₁	CC-5UTR-Int ^C -AG ^C -3UTR-A100-t21-deaKu5g	CC	5UTR	Int ^C -AG ^C	3UTR	100	t21-deaKu5g
14	Int ^C -AG ^C m-OFF ₂₁	CC-5UTR-t21-Int ^C -AG ^C m-3UTR-A120	CC	5UTR-t21	Int ^C -AG ^C m	3UTR	120	-
14m	Int ^C -AG ^C m-OFF ₂₁	CCm-5UTR-t21-Int ^C -AG ^C m-3UTR-A120	CCm	5UTR-t21	Int ^C -AG ^C m	3UTR	120	-
15	PAC	CCm-5UTR-PAC-3UTR-A120	CCm	5UTR	PAC	3UTR	120	-
18	PAC-ON ₂₁	CCm-5UTR-PAC-3UTR-A100-t21-deaKu5g	CCm	5UTR	PAC	3UTR	100	t21-deaKu5g
21	PAC ^N -Int ^N -ON ₂₁	CCm-5UTR-PAC ^N -Int ^N -3UTR-A100-t21-deaKu5g	CCm	5UTR	PAC ^N -Int ^N	3UTR	100	t21-deaKu5g
22	Int ^C -PAC ^C -ON ₂₁	CCm-5UTR-Int ^C -PAC ^C -3UTR-A100-t21-deaKu5g	CCm	5UTR	Int ^C -PAC ^C	3UTR	100	t21-deaKu5g
24	Int ^C -PAC ^C m-OFF ₂₁	CCm-5UTR-t21-Int ^C -PAC ^C m-3UTR-A120	CCm	5UTR-t21	Int ^C -PAC ^C m	3UTR	120	-
26	RF _{A118C}	CC-5UTR-iRFP670 _{A118C} -3UTR-A100	CC	5UTR	iRFP670 _{A118C}	3UTR	100	-
27	RF ^N -Int ^N	CC-5UTR-iRFP ^N -Int ^N -3UTR-A100	CC	5UTR	RF ^N -Int ^N	3UTR	100	-
28	Int ^C -RF ^C	CC-5UTR-Int ^C -iRFP ^C -3UTR-A100	CC	5UTR	Int ^C -RF ^C	3UTR	100	-
29	RF _{A118C} -OFF ₂₁	CC-5UTR-t21-iRFP670 _{A118C} -3UTR-A120	CC	5UTR-t21	iRFP670 _{A118C}	3UTR	120	-
30	RF ^N -Int ^N -OFF ₂₁	CC-5UTR-t21-iRFP ^N -Int ^N -3UTR-A120	CC	5UTR-t21	RF ^N -Int ^N	3UTR	120	-
31	Int ^C -RF ^C -OFF ₂₁	CC-5UTR-t21-Int ^C -iRFP ^C -3UTR-A120	CC	5UTR-t21	Int ^C -RF ^C	3UTR	120	-
32	PAC-ON _{302a}	CCm-5UTR-PAC-3UTR-A100-t302a5p-deaKu5g	CCm	5UTR	PAC	3UTR	100	-
33	PAC ^N -Int ^N -ON _{302a}	CCm-5UTR-PAC ^N -Int ^N -3UTR-A100-t302a5p-deaKu5g	CCm	5UTR	PAC ^N -Int ^N	3UTR	100	t302a5p-deaKu5g
34	Int ^C -PAC ^C -ON _{302a}	CCm-5UTR-Int ^C -PAC ^C -3UTR-A100-t302a5p-deaKu5g	CCm	5UTR	Int ^C -PAC ^C	3UTR	100	t302a5p-deaKu5g
35	Int ^C -PAC ^C m-OFF _{302a}	CCm-5UTR-t302a5p-Int ^C -PAC ^C m-3UTR-A120	CCm	5UTR-t302a5p	Int ^C -PAC ^C m	3UTR	120	-
36	RF-ON _{302a}	CC-5UTR-iRFP670-3UTR-A100-t302a5p-deaKu5g	CC	5UTR	iRFP670 _{A118C}	3UTR	100	t302a5p-deaKu5g
37	Int ^C -AG ^C -ON _{302a}	CC-5UTR-Int ^C -AG ^C -3UTR-A100-t302a5p-deaKu5g	CC	5UTR	Int ^C -AG ^C	3UTR	100	t302a5p-deaKu5g
38	Int ^C -AG ^C m-OFF _{302a}	CC-5UTR-t302a5p-Int ^C -AG ^C m-3UTR-A120	CC	5UTR-t302a5p	Int ^C -AG ^C m	3UTR	120	-
38m	Int ^C -AG ^C m-OFF _{302a}	CCm-5UTR-t302a5p-Int ^C -AG ^C m-3UTR-A120	CCm	5UTR-t302a5p	Int ^C -AG ^C m	3UTR	120	-
39	RF ^N -Int ^N -OFF _{302a}	CC-5UTR-t302a5p-iRFP ^N -Int ^N -3UTR-A120	CC	5UTR-t302a5p	RF ^N -Int ^N	3UTR	120	-
40	AG ^N -Int ^N -OFF ₂₁	CC-5UTR-t21-AG ^N -Int ^N -3UTR-A120	CC	5UTR-t21	AG ^N -Int ^N	3UTR	120	-
40m	AG ^N -Int ^N -OFF ₂₁	CCm-5UTR-t21-AG ^N -Int ^N -3UTR-A120	CCm	5UTR-t21	AG ^N -Int ^N	3UTR	120	-
41	Int ^C -AG ^C -OFF ₂₁	CC-5UTR-t21-Int ^C -AG ^C -3UTR-A120	CC	5UTR-t21	Int ^C -AG ^C	3UTR	120	-
41m	Int ^C -AG ^C -OFF ₂₁	CCm-5UTR-t21-Int ^C -AG ^C -3UTR-A120	CCm	5UTR-t21	Int ^C -AG ^C	3UTR	120	-
42	Int ^C -AG ^C -OFF _{302a}	CC-5UTR-t302a5p-Int ^C -AG ^C m-3UTR-A120	CC	5UTR-t302a5p	Int ^C -AG ^C	3UTR	120	-
42m	Int ^C -AG ^C -OFF _{302a}	CCm-5UTR-t302a5p-Int ^C -AG ^C m-3UTR-A120	CCm	5UTR-t302a5p	Int ^C -AG ^C	3UTR	120	-
43	AG ^N -Int ^N -ON _{302a}	CCm-5UTR-AG ^N -Int ^N -3UTR-A100-t302a5p-deaKu5g	CCm	5UTR	AG ^N -Int ^N	3UTR	100	t302a5p-deaKu5g
44	TKA _{266C}	CCm-5UTR-TKA _{266C} -3UTR-A120	CCm	5UTR	TKA _{266C}	3UTR	120	-

45	TK ^N -Int ^N	CCm-5UTR-TK ^N -Int ^N -3UTR-A120	CCm	5UTR	TK ^N -Int ^N	3UTR	120	-
46	Int ^C -TK ^C	CCm-5UTR-Int ^C -TK ^C -3UTR-A120	CCm	5UTR	Int ^C -TK ^C	3UTR	120	-
47	TKA266C-ON21	CCm-5UTR-TKA266C-3UTR-A100-t21-deaKu5g	CCm	5UTR	TKA266C	3UTR	100	t21-deaKu5g
48	TK ^N -Int ^N -ON21	CCm-5UTR-TK ^N -Int ^N -3UTR-A100-t21-deaKu5g	CCm	5UTR	TK ^N -Int ^N	3UTR	100	t21-deaKu5g
49	Int ^C -TK ^C -ON21	CCm-5UTR-Int ^C -TK ^C -3UTR-A100-t21-deaKu5g	CCm	5UTR	Int ^C -TK ^C	3UTR	100	t21-deaKu5g
50	HPH	CCm-5UTR-HPH-3UTR-A120	CCm	5UTR	HPH	3UTR	120	-
51	HPH ^N -Int ^N	CCm-5UTR-HPH ^N -Int ^N -3UTR-A120	CCm	5UTR	HPH ^N -Int ^N	3UTR	120	-
52	Int ^C -HPH ^C	CCm-5UTR-Int ^C -HPH ^C -3UTR-A120	CCm	5UTR	Int ^C -HPH ^C	3UTR	120	-
53	BSR	CCm-5UTR-BSR-3UTR-A120	CCm	5UTR	BSR	3UTR	120	-
54	BSR ^N -Int ^N	CCm-5UTR-BSR ^N -Int ^N -3UTR-A120	CCm	5UTR	BSR ^N -Int ^N	3UTR	120	-
55	Int ^C -BSR ^C	CCm-5UTR-Int ^C -BSR ^C -3UTR-A120	CCm	5UTR	Int ^C -BSR ^C	3UTR	120	-
56	HPH-ON21	CCm-5UTR-HPH-3UTR-A100-t21-deaKu5g	CCm	5UTR	HPH	3UTR	100	t21-deaKu5g
57	HPH ^N -Int ^N -ON21	CCm-5UTR-HPH ^N -Int ^N -3UTR-A100-t21-deaKu5g	CCm	5UTR	HPH ^N -Int ^N	3UTR	100	t21-deaKu5g
58	Int ^C -HPH ^C -ON21	CCm-5UTR-Int ^C -HPH ^C -3UTR-A100-t21-deaKu5g	CCm	5UTR	Int ^C -HPH ^C	3UTR	100	t21-deaKu5g
59	BSR-ON21	CCm-5UTR-BSR-3UTR-A100-t21-deaKu5g	CCm	5UTR	BSR	3UTR	100	t21-deaKu5g
60	BSR ^N -Int ^N -ON21	CCm-5UTR-BSR ^N -Int ^N -3UTR-A100-t21-deaKu5g	CCm	5UTR	BSR ^N -Int ^N	3UTR	100	t21-deaKu5g
61	Int ^C -BSR ^C -ON21	CCm-5UTR-Int ^C -BSR ^C -3UTR-A100-t21-deaKu5g	CCm	5UTR	Int ^C -BSR ^C	3UTR	100	t21-deaKu5g
63	spCas9 ^{both}	CCm-Kit5UTR-spCas9 ^{both} -Kit3UTR-A120	CCm	5UTR	spCas9 ^{both}	3UTR	120	-
65	NLS-spCas9 ^N -Int ^N	CCm-Kit5UTR-NLS-spCas9 ^N -Int ^N -Kit3UTR-A120	CCm	5UTR	NLS-spCas9 ^N -Int ^N	3UTR	120	-
66	Int ^C -spCas9 ^C	CCm-Kit5UTR-Int ^C -spCas9 ^C -Kit3UTR-A120	CCm	5UTR	Int ^C -spCas9 ^C	3UTR	120	-
67	AG-OFF _{L7Ae}	CCm-Kt-Kit5UTR-hmAG1Q63C-Kit3UTR-A120	CCm	5UTR	hmAG1Q63C	3UTR	120	-
68	AG ^N -Int ^N -OFF _{L7Ae}	CCm-Kt-Kit5UTR-AG ^N -Int ^N -Kit3UTR-A120	CCm	5UTR	AG ^N -Int ^N	3UTR	120	-
69	L7Ae	CCm-Kit5UTR-L7Ae-Kit3UTR-A120	CCm	5UTR	L7Ae	3UTR	120	-
70	AG-OFF _{LIN28A}	CCm-stbC-Kit5UTR-hmAG1Q63C-Kit3UTR-A120	CCm	5UTR	hmAG1Q63C	3UTR	120	-
71	AG ^N -Int ^N -OFF _{LIN28A}	CCm-stbC-Kit5UTR-AG ^N -Int ^N -Kit3UTR-A120	CCm	5UTR	AG ^N -Int ^N	3UTR	120	-
72	LIN28A	CCm-Kit5UTR-LIN28A-Kit3UTR-A120	CCm	5UTR	LIN28A	3UTR	120	-
73	AGQ63C_v2	CCm-Kit5UTR-hmAG1Q63C-Kit3UTR-A120	CCm	5UTR	hmAG1Q63C	3UTR	120	-
74	RFA118C_v2	CCm-Kit5UTR-iRFP670A118C-Kit3UTR-A120	CCm	5UTR	iRFP670A118C	3UTR	120	-
75	AGQ63C-OFF _{302a}	CCm-Kit5UTR-t302a5p-hmAG1Q63C-Kit3UTR-A120	CCm	5UTR-t302a5p	hmAG1Q63C	3UTR	120	-
76	spCas9 ^{both} -ON21	CCm-Kit5UTR-spCas9 ^{both} -Kit3UTR-A100-t21-deaKu5g	CCm	5UTR	spCas9 ^{both}	3UTR	100	t21-deaKu5g
77	NLS-spCas9 ^N -Int ^N -ON21	CCm-Kit5UTR-NLS-spCas9 ^N -Int ^N -Kit3UTR-A100-t21-deaKu5g	CCm	5UTR	NLS-spCas9 ^N -Int ^N	3UTR	100	t21-deaKu5g
78	Int ^C -spCas9 ^C -NLS-ON21	CCm-Kit5UTR-Int ^C -spCas9 ^C -NLS-Kit3UTR-A100-t21-deaKu5g	CCm	5UTR	Int ^C -spCas9 ^C	3UTR	100	t21-deaKu5g
79	AGQ63C, P131S	CCm-Kit5UTR-AGQ63C, P131S-Kit3UTR-A120	CCm	5UTR	AGQ63C, P131S	3UTR	120	-
80	AGQ63C, V153S	CCm-Kit5UTR-AGQ63C, V153S-Kit3UTR-A120	CCm	5UTR	AGQ63C, V153S	3UTR	120	-
81	AGQ63C, G167S	CCm-Kit5UTR-AGQ63C, G167S-Kit3UTR-A120	CCm	5UTR	AGQ63C, G167S	3UTR	120	-
82	AGP131S-aN	CCm-Kit5UTR-AGP131S-aN-Kit3UTR-A120	CCm	5UTR	AGP131S-aN	3UTR	120	-
83	AGP131S-aC	CCm-Kit5UTR-AGP131S-aC-Kit3UTR-A120	CCm	5UTR	AGP131S-aC	3UTR	120	-
84	AGP131S-bN	CCm-Kit5UTR-AGP131S-bN-Kit3UTR-A120	CCm	5UTR	AGP131S-bN	3UTR	120	-
85	AGP131S-bC	CCm-Kit5UTR-AGP131S-bC-Kit3UTR-A120	CCm	5UTR	AGP131S-bC	3UTR	120	-
86	AGV153S-aN	CCm-Kit5UTR-AGV153S-aN-Kit3UTR-A120	CCm	5UTR	AGV153S-aN	3UTR	120	-
87	AGV153S-aC	CCm-Kit5UTR-AGV153S-aC-Kit3UTR-A120	CCm	5UTR	AGV153S-aC	3UTR	120	-
88	AGV153S-bN	CCm-Kit5UTR-AGV153S-bN-Kit3UTR-A120	CCm	5UTR	AGV153S-bN	3UTR	120	-
89	AGV153S-bC	CCm-Kit5UTR-AGV153S-bC-Kit3UTR-A120	CCm	5UTR	AGV153S-bC	3UTR	120	-
90	AGG167S-aN	CCm-Kit5UTR-AGG167S-aN-Kit3UTR-A120	CCm	5UTR	AGG167S-aN	3UTR	120	-
91	AGG167S-aC	CCm-Kit5UTR-AGG167S-aC-Kit3UTR-A120	CCm	5UTR	AGG167S-aC	3UTR	120	-
92	AGG167S-bN	CCm-Kit5UTR-AGG167S-bN-Kit3UTR-A120	CCm	5UTR	AGG167S-bN	3UTR	120	-
93	AGG167S-bC	CCm-Kit5UTR-AGG167S-bC-Kit3UTR-A120	CCm	5UTR	AGG167S-bC	3UTR	120	-
96	spCas9 ^{both} -ON _{302a}	CCm-Kit5UTR-spCas9 ^{both} -Kit3UTR-A100-t302a-deaKu5g	CCm	5UTR	spCas9 ^{both}	3UTR	100	t302a5p-deaKu5g
97	NLS-spCas9 ^N -Int ^N -ON _{302a}	CCm-Kit5UTR-NLS-spCas9 ^N -Int ^N -Kit3UTR-A100-t302a-deaKu5g	CCm	5UTR	NLS-spCas9 ^N -Int ^N	3UTR	100	t302a5p-deaKu5g
98	Int ^C -spCas9 ^C -NLS-ON _{302a}	CCm-Kit5UTR-Int ^C -spCas9 ^C -NLS-Kit3UTR-A100-t302a-deaKu5g	CCm	5UTR	Int ^C -spCas9 ^C -NLS	3UTR	100	t302a5p-deaKu5g

99	Npu^C-AG^{mid}-Ssp^N	CCm-Kit5UTR-Npu ^C -AG ^{mid} -Ssp ^N -Kit3UTR-A ₁₂₀	CCm	5UTR	Npu ^C -AG ^{mid} -Ssp ^N	3UTR	120	-
100	Npu^C-AG^{mid}-Ssp^N-OFF_{302a}	CCm-5UTR-t302a5p-Npu ^C -AG ^{mid} -Ssp ^N -3UTR-A ₁₂₀	CCm	5UTR-t302a5p	Npu ^C -AG ^{mid} -Ssp ^N	3UTR	120	-
101	AG^{P131S}-bC-OFF₂₀₆	CCm-5UTR-t206-AG ^{P131S} -bC-3UTR-A ₁₂₀	CCm	5UTR-t206	AG ^{P131S} -bC	3UTR	120	-
102	AG^N-Int^N	CCm-5UTR-AG ^N -Int ^N -3UTR-A ₁₂₀	CCm	5UTR	AG ^N -Int ^N	3UTR	120	-
103	Npu^C-AG^{mid}-Ssp^N-ON_{302a}	CCm-5UTR-Npu ^C -AG ^{mid} -Ssp ^N -3UTR-A ₁₀₀ -t302a5p-deaKu5g	CCm	5UTR	Npu ^C -AG ^{mid} -Ssp ^N	3UTR	100	t302a5p-deaKu5g
104	AG^{P131S}-bC-ON₂₀₆	CCm-5UTR-AG ^{P131S} -bC-3UTR-A ₁₀₀ -t206-deaKu5g	CCm	5UTR	AG ^{P131S} -bC	3UTR	100	t206-deaKu5g
105	AG^{mid}-m-Npu^N-OFF₂₁	CCm-5UTR-t21-AG ^{mid} -m-Npu ^N -3UTR-A ₁₂₀	CCm	5UTR-t21	AG ^{mid} -m-Npu ^N	3UTR	120	-
106	Npu^C-AG^{mid}-Ssp^N-OFF_{302a}	CCm-5UTR-t302a5p-Npu ^C -AG ^{mid} -m-Ssp ^N -3UTR-A ₁₂₀	CCm	5UTR-t302a5p	Npu ^C -AG ^{mid} -m-Ssp ^N	3UTR	120	-
107	Ssp^C-AG^{mid}-m-OFF₂₀₆	CCm-5UTR-t206-Ssp ^C -AG ^{mid} -m-3UTR-A ₁₂₀	CCm	5UTR-t206	Ssp ^C -AG ^{mid} -m	3UTR	120	-

Supplementary Table 3

Detailed information on the conditions for cell experiments.

Fig.	Experiment	Plate type	Medium volume (μl)	Cell type	Cell number per well	miRNA mimic or Inhibitor	Conc. (nM)	mRNA	Volume (ng)	Antibiotics
2b, c, d	split-AG ON switch	24 well plate	500	293FT	75,000	miR-21-5p mimic NC mimic	4 nM 4 nM	11: AG ^{Q63C} -ON ₂₁ 12: AG ^N -Int ^N -ON ₂₁ 13: Int ^C -AG ^C -ON ₂₁ 14: Int ^C -AG ^C m-OFF ₂₁ 26: RF ^{A118C} (internal control)	50 50 50 50 x1 = 50, x4 = 200	-
3b	split-PAC ON switch	96 well plate	100	HeLa	10,000	miR-21-5p inhibitor NC inhibitor	4 nM 4 nM	15: PAC 18: PAC-ON ₂₁ 21: PAC ^N -Int ^N -ON ₂₁ 22: Int ^C -PAC ^C -ON ₂₁ 24: Int ^C -PAC ^C m-OFF ₂₁	25 25 25 25 100	Puromycin: 2 μg/ml
3d	split-HPH ON switch	96 well plate	100	HeLa	10,000	miR-21-5p inhibitor NC inhibitor	4 nM 4 nM	24: Int ^C -PAC ^C m-OFF ₂₁ 50: HPH 56: HPH-ON ₂₁ 57: HPH ^N -Int ^N -ON ₂₁ 58: Int ^C -HPH ^C -ON ₂₁	100 25 25 25 25	Hygromycin B: 500 μg/ml
3f	split-BSR ON switch	96 well plate	100	HeLa	10,000	miR-21-5p inhibitor NC inhibitor	4 nM 4 nM	24: Int ^C -PAC ^C m-OFF ₂₁ 53: BSR 59: BSR-ON ₂₁ 60: BSR ^N -Int ^N -ON ₂₁ 61: Int ^C -BSR ^C -ON ₂₁	100 25 25 25 25	Blasticidin S: 100 μg/ml
3h	split-TK ON switch	96 well plate	100	HeLa	10,000	miR-21-5p inhibitor NC inhibitor	4 nM 4 nM	14: Int ^C -AG ^C m-OFF ₂₁ 44: TK ^{A266C} 47: TK ^{A266C} -ON ₂₁ 48: TK ^N -Int ^N -ON ₂₁ 49: Int ^C -TK ^C -ON ₂₁	100 25 25 25 25	Ganciclovir: 10 μg/ml
4c, d	Hela selection from the mixture with 293FT	96 well plate	100	HeLa ^{hmlA} G1-M9 HEK293 FTIRFP670-M9	10,000 2,000			14: Int ^C -AG ^C m-OFF ₂₁ 53: BSR 59: BSR-ON ₂₁ 60: BSR ^N -Int ^N -ON ₂₁ 61: Int ^C -BSR ^C -ON ₂₁	200 25 25 25 25	Blasticidin S: 100 μg/ml
5b	miR-21-5p-responsive split-Cas9 ON switch in HeLaEGFP ± miR-21-5p	24 well plate	500	HeLaEGFP	20,000	miR-21-5p inhibitor NC inhibitor	4 nM 4 nM	14: Int ^C -AG ^C m-OFF ₂₁ 63: spCas9 ^{both} 76: spCas9 ^{both} -ON ₂₁ 77: NLS-spCas9 ^N -Int ^N -ON ₂₁ 78: Int ^C -spCas9 ^C -NLS-ON ₂₁	x3 = 300, x6 = 600 100 100 100 100	-
5c	miR-302a-5p-responsive split-Cas9 ON switch in hiPSC ^{EGFP} ± miR-302a-5p	24 well plate	500	hiPSC ^{EGFP} _P	20,000	miR-302a-5p inhibitor NC inhibitor	4 nM 4 nM	38m: Int ^C -AG ^C m-OFF _{302a} 63: spCas9 ^{both} 96: spCas9 ^{both} -ON _{302a} 97: NLS-spCas9 ^N -Int ^N -ON _{302a} 98: Int ^C -spCas9 ^C -NLS-ON _{302a}	x3 = 300, x6 = 600 100 100 100 100	-
5e	DMDex45 editing by miR-21-5p-responsive split-Cas9 ON switch in HEK293TEGFP ± miR-21a-5p	24 well plate	500	HEK293TEGFP	40,000	miR-21-5p inhibitor miR-21-5p mimic	4 nM 4 nM	14m: Int ^C -AG ^C m-OFF ₂₁ 63: spCas9 ^{both} 76: spCas9 ^{both} -ON ₂₁ 77: NLS-spCas9 ^N -Int ^N -ON ₂₁ 78: Int ^C -spCas9 ^C -NLS-ON ₂₁	x3 = 300, x6 = 600 100 100 100 100	-
6b, c, d	Toggle-like system	24 well plate	500	293FT	75,000	miR-21-5p mimic	titrated as	11: AG ^{Q63C} -ON ₂₁ 12: AG ^N -Int ^N -ON ₂₁	100 100	-

							shown in figure	13: Int ^C -AG ^C -ON ₂₁ 29: RF ^{A118C} -OFF ₂₁ 30: RF ^N -Int ^N -OFF ₂₁ 31: Int ^C -RF ^C -OFF ₂₁	100 100 100 100	
7b	2-input system [00]	24 well plate	500	293FT	75,000	miR-21-5p mimic miR-302a- 5p mimic	4 nM 4 nM	12: AG ^N -Int ^N -ON ₂₁ 14: Int ^C -AG ^C m-OFF ₂₁ 26: RF ^{A118C} (internal control) 37: Int ^C -AG ^C -ON _{302a} 39: RF ^N -Int ^N -OFF _{302a}	100 300 100 200 300	-
	2-input system [10]	24 well plate	500	293FT	75,000	miR-21-5p mimic miR-302a- 5p mimic	4 nM 4 nM	26: RF ^{A118C} (internal control) 40: AG ^N -Int ^N -OFF ₂₁ 42: Int ^C -AG ^C -OFF _{302a}	100 100 100	-
	2-input system [01]	24 well plate	500	293FT	75,000	miR-21-5p mimic miR-302a- 5p mimic	4 nM 4 nM	12: AG ^N -Int ^N -ON ₂₁ 14: Int ^C -AG ^C m-OFF ₂₁ 26: RF ^{A118C} (internal control) 42: Int ^C -AG ^C -OFF _{302a}	100 300 100 100	-
	2-input system [01]	24 well plate	500	293FT	75,000	miR-21-5p mimic miR-302a- 5p mimic	4 nM 4 nM	26: RF ^{A118C} (internal control) 38: Int ^C -AG ^C m-OFF _{302a} 41: Int ^C -AG ^C -OFF ₂₁ 43: AG ^N -Int ^N -ON _{302a}	100 300 100 200	-
8b	miR-21/L7Ae- NOR gate	24 well plate	500	293FT	75,000	miR-21-5p mimic	4 nM	26: RF ^{A118C} (internal control) 41m: Int ^C -AG ^C -OFF ₂₁ 68: AG ^N -Int ^N -OFF _{L7Ae} 69: L7Ae 73: AGQ63C_v2	100 100 100 20 100	-
	miR- 302a/LIN28A- NOR gate	24 well plate	500	293FT	75,000	miR-302a- 5p mimic	4 nM	26: RF ^{A118C} (internal control) 42m: Int ^C -AG ^C -OFF _{302a} 71: AG ^N -Int ^N -OFF _{LIN28A} 72: LIN28A 73: AGQ63C_v2	100 100 200 600 100	-
8c	miR- 302a/LIN28A- NOR gate in iPSC	24 well plate	500	293FT hiPSC	75,000 75,000			42m: Int ^C -AG ^C -OFF _{302a} 70: AG-OFF _{LIN28A} 71: AG ^N -Int ^N -OFF _{LIN28A} 74: RF ^{A118C} _v2 75: AGQ63C -OFF _{302a}	100 200 200 100 100	-
8e	miR- 21/302a/206 NOR gate	24 well plate	500	293FT	75,000	miR-21-5p mimic miR-302a- 5p mimic miR-206 mimic	4 nM 4 nM 4 nM	26: RF ^{A118C} (internal control) 40m: AG ^N -Int ^N -OFF ₂₁ 100: Npu ^C -AG ^{mid} -Ssp ^N - OFF _{302a} 101: AGP131S-bC-OFF ₂₀₆	50 50 50 50	-
	miR- 21/302a/206 AND gate	24 well plate	500	293FT	75,000	miR-21-5p mimic miR-302a- 5p mimic miR-206 mimic	4 nM 4 nM 4 nM	12m: AG ^N -Int ^N -ON ₂₁ 26: RF ^{A118C} (internal control) 103: Npu ^C -AG ^{mid} -Ssp ^N - ON _{302a} 104: AGP131S-bC-ON ₂₀₆ 105: AG ^{mid} m-Npu ^N - OFF ₂₁ 106: Npu ^C -AG ^{mid} m- Ssp ^N -OFF _{302a} 107: Ssp ^C -AG ^{mid} m- OFF ₂₀₆	50 50 50 50 150 150 600	-
S2	investigation of extra sequence	24 well plate	500	293FT	100,000	miR-21-5p mimic NC mimic	4 nM 4 nM	S1: 21switch_ctrl S2: 21switch_a S3: 21switch_d S4: 21switch_ad S5: 21switch_da R: iRFP	100 100 100 100 100 100	-
S3			500	293FT	100,000			1: AGQ63C	100	-

Supplementary Table 4

Statistics and *P*-values.

Figure	Name	Condition #1	Condition #2	Value	Statistical analysis	<i>P</i> -value
Fig.2b	-	AGQ63C-ON21	AGN-IntN-ON21 IntC-AGC-ON21	ON/OFF ratio of relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	0.00999
	-	AGN-IntN-ON21 IntC-AGC-ON21	AGN-IntN-ON21 IntC-AGC-ON21 IntC-AGCm-OFF21 x1	ON/OFF ratio of relative hmAG2 intensity [a.u.]	Two-sided Welch's t-test	0.00270
	-	AGN-IntN-ON21 IntC-AGC-ON21 IntC-AGCm-OFF21 x1	AGN-IntN-ON21 IntC-AGC-ON21 IntC-AGCm-OFF21 x4	ON/OFF ratio of relative hmAG3 intensity [a.u.]	Two-sided Welch's t-test	0.00179
	-	AGN-IntN-ON21 IntC-AGC-ON21	AGN-IntN-ON21 IntC-AGC-ON21 IntC-AGCm-OFF21 x4	ON/OFF ratio of relative hmAG4 intensity [a.u.]	Two-sided Welch's t-test	0.00122
Fig.3b	Mock	NC inhibitor	miR-21-5p inhibitor	Survival rate (Puro+/Puro-) [%]	Two-sided Welch's t-test	0.39970
	PAC mRNA	NC inhibitor	miR-21-5p inhibitor	Survival rate (Puro+/Puro-) [%]	Two-sided Welch's t-test	0.38825
	PAC-ON21	NC inhibitor	miR-21-5p inhibitor	Survival rate (Puro+/Puro-) [%]	Two-sided Welch's t-test	0.02450
	PACN-IntN-ON21 IntC-PACC-ON21 IntC-PACCm-OFF21	NC inhibitor	miR-21-5p inhibitor	Survival rate (Puro+/Puro-) [%]	Two-sided Welch's t-test	4.38E-05
Fig.3d	Mock	NC inhibitor	miR-21-5p inhibitor	Survival rate (Hygro+/Hygro-) [%]	Two-sided Welch's t-test	0.71901
	HPH mRNA	NC inhibitor	miR-21-5p inhibitor	Survival rate (Hygro+/Hygro-) [%]	Two-sided Welch's t-test	0.61318
	HPH-ON21	NC inhibitor	miR-21-5p inhibitor	Survival rate (Hygro+/Hygro-) [%]	Two-sided Welch's t-test	0.63277
	HPHN-IntN-ON21 IntC-HPHC-ON21 IntC-PACCm-OFF21	NC inhibitor	miR-21-5p inhibitor	Survival rate (Hygro+/Hygro-) [%]	Two-sided Welch's t-test	0.00040
Fig.3f	Mock	NC inhibitor	miR-21-5p inhibitor	Survival rate (Blast+/Blast-) [%]	Two-sided Welch's t-test	0.80731
	BSR mRNA	NC inhibitor	miR-21-5p inhibitor	Survival rate (Blast+/Blast-) [%]	Two-sided Welch's t-test	0.87472
	BSR-ON21	NC inhibitor	miR-21-5p inhibitor	Survival rate (Blast+/Blast-) [%]	Two-sided Welch's t-test	0.42634
	BSRN-IntN-ON21 IntC-BSRC-ON21 IntC-PACCm-OFF21	NC inhibitor	miR-21-5p inhibitor	Survival rate (Blast+/Blast-) [%]	Two-sided Welch's t-test	0.00081
Fig.3h	Mock	NC inhibitor	miR-21-5p inhibitor	Survival rate (GCV+/GCV-) [%]	Two-sided Welch's t-test	0.73695
	TKA266C mRNA	NC inhibitor	miR-21-5p inhibitor	Survival rate (GCV+/GCV-) [%]	Two-sided Welch's t-test	0.97956
	TKA266C-ON21	NC inhibitor	miR-21-5p inhibitor	Survival rate (GCV+/GCV-) [%]	Two-sided Welch's t-test	0.02819
	TKN-IntN-ON21 IntC-TKC-ON21 IntC-AGCm-OFF21	NC inhibitor	miR-21-5p inhibitor	Survival rate (GCV+/GCV-) [%]	Two-sided Welch's t-test	0.00494
Fig. 5b	Cas9 mRNA	sgRNA+, NC inhibitor	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.95966
	Cas9-ON21	sgRNA+, NC inhibitor	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.03185
	Cas9N-IntN-ON21 IntC-Cas9C-ON21	sgRNA+, NC inhibitor	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.00052
	Cas9N-IntN-ON21 IntC-Cas9C-ON21 IntC-AGCm-OFF21 x3	sgRNA+, NC inhibitor	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.00089
	Cas9N-IntN-ON21 IntC-Cas9C-ON21 IntC-AGCm-OFF21 x6	sgRNA+, NC inhibitor	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.00029
Fig. 5c	Cas9 mRNA	sgRNA+, NC inhibitor	sgRNA+, miR-302a-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.54650
	Cas9-ON302a	sgRNA+, NC inhibitor	sgRNA+, miR-302a-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.73453
	Cas9N-IntN-ON302a IntC-Cas9C-ON302a	sgRNA+, NC inhibitor	sgRNA+, miR-302a-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.59490

	Cas9N-IntN-ON302a IntC-Cas9C-ON302a IntC-AGCm-OFF302a x3	sgRNA+, NC inhibitor	sgRNA+, miR-302a-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	1.71E-05
	Cas9N-IntN-ON302a IntC-Cas9C-ON302a IntC-AGCm-OFF302a x6	sgRNA+, NC inhibitor	sgRNA+, miR-302a-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.00108
Fig. 5e	Cas9 mRNA	sgRNA+, miR-21-5p mimic	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.95492
	Cas9-ON21	sgRNA+, miR-21-5p mimic	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.01016
	Cas9N-IntN-ON21 IntC-Cas9C-ON21	sgRNA+, miR-21-5p mimic	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.00426
	Cas9N-IntN-ON21 IntC-Cas9C-ON21 IntC-AGCm-OFF21 x3	sgRNA+, miR-21-5p mimic	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.00729
	Cas9N-IntN-ON21 IntC-Cas9C-ON21 IntC-AGCm-OFF21 x6	sgRNA+, miR-21-5p mimic	sgRNA+, miR-21-5p inhibitor	EGFP-positive population [%]	Two-sided Welch's t-test	0.01022
Fig.6b	miR-21-5p mimic 0 nM	Normal toggle-like system	Split toggle-like system	Normalized fluorescence intensity (hmAG1)	Two-sided Welch's t-test	0.00202
	miR-21-5p mimic 2 nM	Normal toggle-like system	Split toggle-like system	Normalized fluorescence intensity (iRFP670)	Two-sided Welch's t-test	0.02491
Fig. 7b	NOR	[00]	[10]	Normalized hmAG1 intensity	Two-sided Dunnett's test	3.72E-11
		[00]	[01]			4.10E-11
		[00]	[11]			2.10E-13
	A AND NOT B	[10]	[00]	Normalized hmAG1 intensity	Two-sided Dunnett's test	6.31E-11
		[10]	[01]			1.19E-13
		[10]	[11]			3.83E-10
	NOT A AND B	[01]	[00]	Normalized hmAG1 intensity	Two-sided Dunnett's test	2.99E-10
		[01]	[10]			5.41E-12
		[01]	[11]			7.02E-12
	AND	[11]	[00]	Normalized hmAG1 intensity	Two-sided Dunnett's test	2.91E-09
		[11]	[10]			2.26E-10
		[11]	[01]			1.08E-08
Fig.8b	L7Ae/miR-21-NOR	[-, -]	[miR-21-5p, -]	Normalized hmAG1 intensity	Two-sided Dunnett's test	4.98E-12
		[-, -]	[-, L7Ae]			2.47E-10
		[-, -]	[miR-21-5p, L7Ae]			2.82E-15
	LIN28A/miR-302a-NOR	[-, -]	[miR-302a-5p, -]	Normalized hmAG1 intensity	Two-sided Dunnett's test	1.53E-13
		[-, -]	[-, LIN28A]			9.74E-09
		[-, -]	[miR-302a-5p, LIN28A]			7.33E-13
Fig.8c	-	AGQ63C-OFF302a	AGQ63C- NOR302a/LIN28A	Fold change in relative hmAG intensity (293FT/hiPSC)	Two-sided Welch's t-test	0.01018
	-	AGQ63C-OFFLIN28A	AGQ63C- NOR302a/LIN28A	Fold change in relative hmAG intensity (293FT/hiPSC)	Two-sided Welch's t-test	0.00842
Fig.8e	miR-21/302a/206 NOR gate	[000]	[100]	Normalized hmAG1 intensity	Two-sided Dunnett's test	1.88E-12
		[000]	[010]			3.04E-13
		[000]	[001]			6.44E-10
		[000]	[110]			9.21E-10
		[000]	[011]			3.13E-09
		[000]	[101]			3.31E-10
		[000]	[111]			6.96E-12
	miR-21/302a/206 AND gate	[111]	[000]	Normalized hmAG1 intensity	Two-sided Dunnett's test	1.19E-07
		[111]	[100]			3.72E-07
		[111]	[010]			8.54E-08
		[111]	[001]			1.79E-07
		[111]	[110]			3.17E-07
		[111]	[011]			1.63E-07
		[111]	[101]			6.83E-07
Fig.S2	-A100	miR-21-5p mimic	NC mimic	Relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	0.89212
	-A100-t21-aK-u5g	miR-21-5p mimic	NC mimic	Relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	0.00536

	-A100-t21-DE-u5g	miR-21-5p mimic	NC mimic	Relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	2.68E-05
	-A100-t21-aK-DE-u5g	miR-21-5p mimic	NC mimic	Relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	0.00699
	-A100-t21-DE-aK-u5g	miR-21-5p mimic	NC mimic	Relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	0.00121
	-	-A100-t21-DE-u5g	-A100-t21-DE-aK-u5g	ON/OFF ratio of relative hmAG1 intensity [a.u.]	Two-sided Welch's t-test	0.63925
Fig.S6c (HeLa)	Puromycin (+)	PAC mRNA	PAC-ON302a	Viability [%]	Two-sided Welch's t-test	0.46504
	Puromycin (+)	PAC mRNA	PACN-IntN-ON302a IntC-PACC-ON302a IntC-PACCM-OFF302a x4	Viability [%]	Two-sided Welch's t-test	0.00108
Fig.S6c (hiPSC)	Puromycin (+)	PAC mRNA	PAC-ON302a	Viability [%]	Two-sided Welch's t-test	0.47986
	Puromycin (+)	PAC mRNA	PACN-IntN-ON302a IntC-PACC-ON302a IntC-PACCM-OFF302a x4	Viability [%]	Two-sided Welch's t-test	0.67027
Fig.S11b	-	AGP131S-bN + bC	AGV153S-bN + bC	Normalized hmAG1 intensity	Two-sided Welch's t-test	0.00043
	-	AGV153S-bN + bC	AGG167S-bN + bC	Normalized hmAG1 intensity	Two-sided Welch's t-test	0.10330
	-	AGP131S-bN + bC	AGG167S-bN + bC	Normalized hmAG1 intensity	Two-sided Welch's t-test	0.00209
Fig.S12	-	AGQ63C	AGQ63C, G65A	Viability [%]	Two-sided Welch's t-test	0.97599
	-	AGQ63C	AGN-IntN IntC-AGC	Viability [%]	Two-sided Welch's t-test	0.68266
	-	AGN-IntN IntC-AGC	AGN-IntN IntC-AGCm	Viability [%]	Two-sided Welch's t-test	0.51423

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