

SARS-CoV-2 Nsp1-Resistant Modified RNA for the Creation of Nsp1-Responsive Systems

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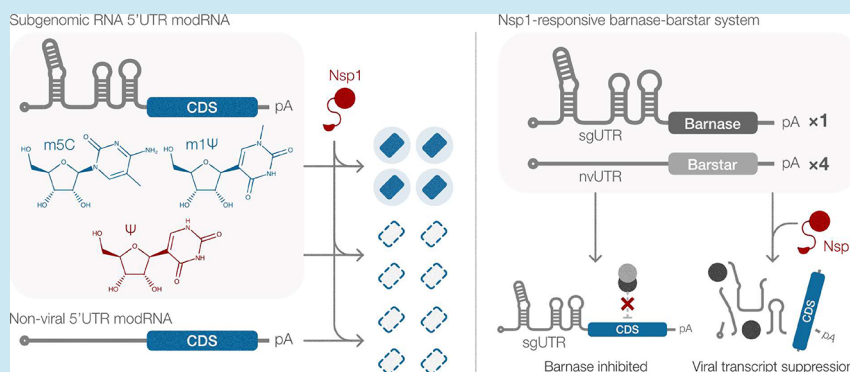
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ABSTRACT: Modified RNA (modRNA) facilitates the introduction of complex synthetic genetic circuits into cells without the risk of genomic integration, opening up the implementation of synthetic circuits as therapeutics. However, the number of protein-RNA interfaces that are suitable for the construction of protein-responsive modRNA switches as well as the lack of protein-responsive exclusive selector systems stifles the development of RNA-based synthetic circuits. Here, we present the creation of a modRNA capable of resisting the effects of Nsp1 for the reliable expression of its coding sequence. Using both the subgenomic viral RNA 5'UTR and two modified nucleosides, we observed efficient exogenous protein expression even in Nsp1-transfected cells. To demonstrate its utility, we developed a barnase-barstar system capable of conditional transcript suppression in the presence of Nsp1. Altogether, the resistance to Nsp1-mediated translational suppression and the resulting Nsp1-sensing system we present in this study provide an invaluable opportunity to develop a new class of protein-sensing systems for the construction of more complex RNA-based genetic circuits.

KEYWORDS: SARS-CoV-2 Nsp1, modRNA, modified nucleosides, translational control, barnase, 5'UTR

In recent years, RNA-based post-transcriptional genetic circuits have received attention due to their potential of performing complex, input-dependent biological responses with minimal safety risk from genomic integration.^{1,2} This idea has been made possible due to the discovery and research in modified ribonucleosides, which allow the synthesis of efficiently translating, low-immunogenicity modified RNA (modRNA).³ Research interest in modRNA in general is further boosted by the successful rollout of mRNA vaccines during the COVID-19 pandemic, which utilize modRNA.⁴ One such component of RNA-based genetic circuits is RNA-protein interfaces, in which the binding or interaction of RNA-binding proteins (RBP) with their respective aptamer-containing mRNA, also called 'switches', controls the expression of the gene encoded by the mRNA.^{5–8} Due to the specificity of the RBP-aptamer pair, orthogonal switches can be constructed, which allow the development of logic circuits.^{9,10} However, most of the available RBP-responsive systems invert the input, in which the binding of the RBP impedes the translation of the mRNA. In contrast, there are no

examples of 'exclusive selectors', in which the expression of all but the target gene is hampered. While there exists noninverting ON-type RBP-responsive switches, which can be combined with OFF-switches expressing other genes to mimic the behavior of exclusive selectors, these systems rely on bulky fusions of such RBPs with translational activators.^{11,12} To complicate matters, even if such exclusive selector RBP-aptamer pairs are found, the incorporation of modified nucleosides may hamper the performance of RBP-responsive mRNA switches due to alterations of binding dynamics.¹ The development of more RBP-aptamer pairings in general and the engineering of exclusive selector RBP-responsive switches in

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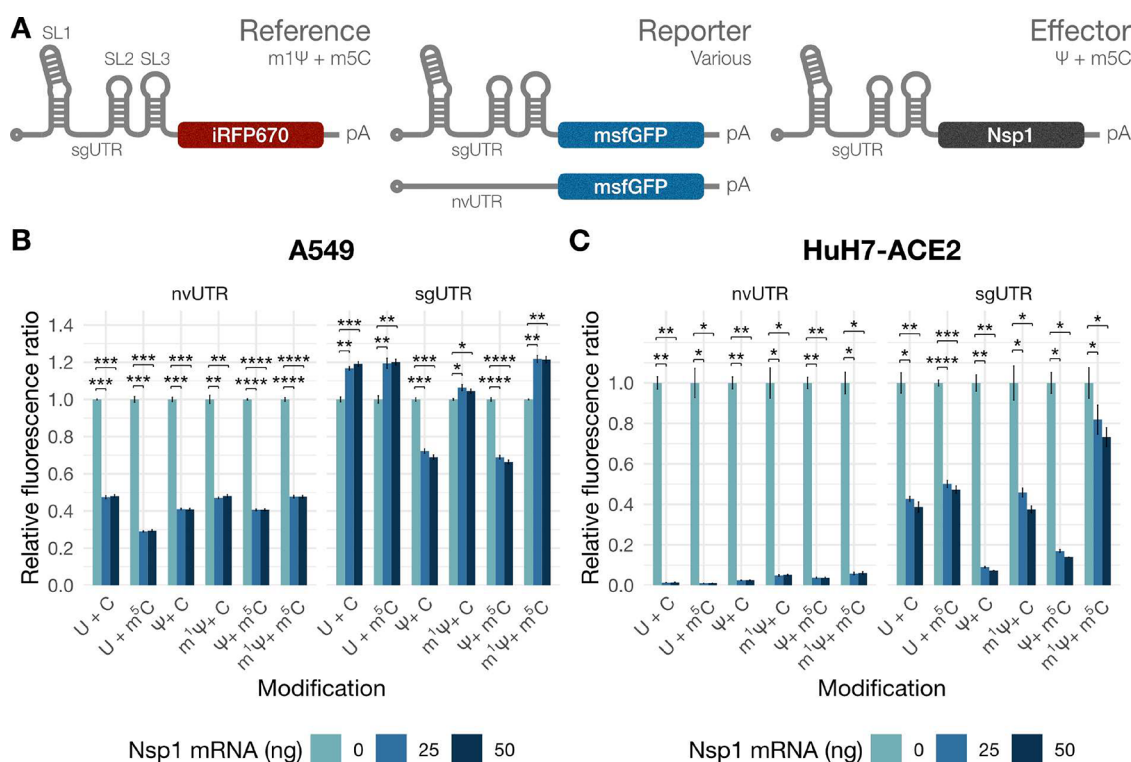


Figure 1. SARS-CoV-2 nsp1 responds differently to sgUTR-mRNAs synthesized with various combinations of modified nucleosides. (A) Illustration of the reporter mRNAs. (B and C) Median GFP/iRFP670 fluorescence intensity ratio of A549 (B) and HuH-7-ACE2 (C) cells cotransfected with nvUTR/sgUTR-GFP mRNAs synthesized with different modified nucleoside combinations, sgUTR-iRFP670 mRNA, and varying amounts of nsp1 mRNA. All experiments were performed in biological triplicates. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

particular that are compatible with modRNA would allow the construction of complex RNA-based genetic circuits.

Coincidentally, such an exclusive selector pairing has been discovered in SARS-CoV-2, the principal cause of COVID-19. It has been observed that host transcript expression in cells infected with the virus is suppressed, while viral RNA, both genomic and subgenomic, remains translatable.¹³ It has since been discovered that this phenomenon is due to the effect of nonstructural protein 1 (Nsp1), which is a translational suppressor that binds to the 40S ribosomal subunit mRNA entry channel.^{14,15} While host mRNAs are inhibited from being translated and subsequently degraded, viral RNAs are able to bypass Nsp1 due to sequence features in their 5'-leader region, effectively revealing the role of Nsp1 as a translational gatekeeper.¹⁶ This raises the possibility of using the Nsp1-viral RNA leader pair as the basis for the construction of exclusive selector protein-responsive mRNA switches. However, previous studies were performed with either plasmid transfection or mRNA synthesized with native bases, so the effects of modified nucleoside incorporation remain unclear.^{16–18}

To determine whether modRNA with viral 5'-leader sequence features is able to tolerate SARS-CoV-2 Nsp1, we devised a reporter assay to quantify the translatability of mRNAs with modified nucleosides under the influence of Nsp1. We synthesized synthetic reporter mRNAs encoding msfGFP containing either the SARS-CoV-2 subgenomic RNA 5'UTR (sgUTR) or a nonviral origin 5'UTR (nvUTR) in their 5'UTRs with different combinations of modified nucleosides, including 5-methylcytosine (m⁵C), pseudouridine (Ψ), and N1-methylpseudouridine (m¹ Ψ), which are often incorporated

into modRNA to suppress cellular immune responses.^{19–21}

These reporter mRNAs were cotransfected with a reference modRNA encoding iRFP670 and an effector modRNA encoding Nsp1 (Figure 1A). We discovered that nvUTR-containing modRNA expression in A549, a lung-derived cell line used in SARS-CoV-2 experiments,²² was suppressed by Nsp1, regardless of the modified bases used (Figures 1B, S1A,B and S2A). Similar results were observed using the human hepatoma-derived hepatocyte line HuH7 with ACE2 overexpression (HuH7-ACE2), previously used for SARS-CoV-2 challenge experiments (Figures 1C and S2B). In contrast, sgUTR-containing modRNAs did not undergo translational suppression, except for pseudouridine (Ψ)-containing variants (Figures 1B and S2A). The slight increase in expression in the presence of Nsp1 was likely due to the reallocation of translational resources from host mRNAs to sgUTR-mRNAs. In HuH7-ACE2 cells, all sgUTR modRNA translation was suppressed, although the suppressive effect was weaker than nvUTR modRNAs. Among them, the Ψ -containing modRNA showed the most substantial decline (Figures 1C, S1C,D and S2B). Among all nucleoside combinations, we found that N1-methylpseudouridine (m¹ Ψ) and 5-methylcytosine (m⁵C) incorporation provided the most Nsp1-resistant sgUTR modRNA (Figures 1C and S2B). Considering that the ability of the viral RNA 5'-leader to bypass Nsp1 has been shown to be dependent on its interaction with Nsp1,^{17,23} our observations suggest that Ψ incorporation may disrupt or weaken this specific interaction, whereas m¹ Ψ and m⁵C may strengthen it. Thus, using the combination of m¹ Ψ and m⁵C, we can design modRNAs capable of maintaining at least some

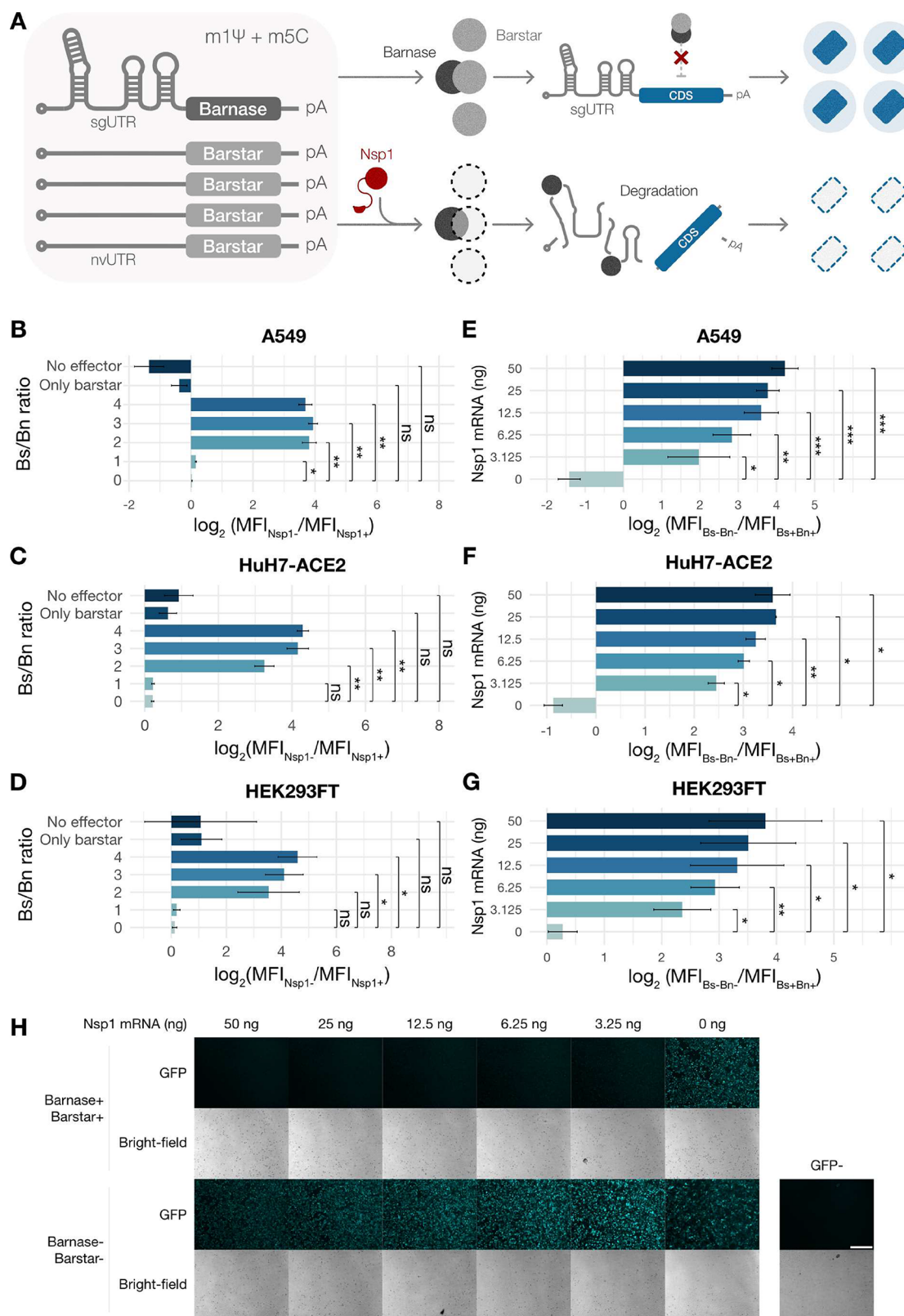


Figure 2. Optimization and characterization of the nsp1-responsive barnase-barstar (Bn-Bs) mRNA system. (A) Illustration of the nsp1-responsive Bn-Bs mRNA system. The modRNAs for the system were synthesized with m1Ψ and m5C. (B–D) Optimization of the Bs/Bn ratio in A549 (B), HuH-7-ACE2 (C), and HEK293FT (D) cell lines transfected with Nsp1 mRNA. (E–G) Median fluorescence GFP intensity ratio of cells transfected with a 4:1 Bs/Bn mRNA ratio against varying amounts of nsp1 mRNA in A549 (E), HuH-7-ACE2 (F), and HEK293FT (G) cells. (H) Representative GFP fluorescence and bright-field images of cells transfected with sgUTR-GFP, a 4:1 nvUTR-Bs/sGUTR-Bn mRNA ratio, and various amounts of nsp1 in A549 cells. Values are normalized to no-nsp1 (0 ng) control. All experiments were performed in biological triplicates. Significance levels: **P* < 0.05, ***P* < 0.01. Scale bar: 500 μm.

degree of resistance against SARS-CoV-2 Nsp1-mediated translational suppression.

To demonstrate the utility of Nsp1-resistant m1 Ψ - and m5C-substituted modRNAs, we designed an Nsp1-responsive OFF switch capable of shutting down the translation of RNAs with SARS-CoV-2 5'-leader features when Nsp1 is present. We employed the barnase-barstar toxin-antitoxin pair: barnase (Bn) is a lethal ribonuclease suppressible by barstar (Bs). The Bn-Bs pair was previously used in a microRNA (miRNA)-responsive mRNA switch system intended for cell type-specific killing, based on the concept that the activity of certain miRNA suppresses and elevates Bs and Bn expression in an opposing manner, thus shifting the Bn-Bs balance to induce transcript degradation in cells.³² However, it has been unclear whether the Bn-Bs system also senses proteins of interest (e.g., Nsp1) to regulate their own expression. Thus, we aimed to replicate this dynamic with Nsp1 by combining sgUTR-Bn modRNA with excess nvUTR-Bs modRNA (Figure 2A). Under normal conditions, while Bn is inactivated due to excessive Bs expression, Nsp1 causes the selective translational suppression of nvUTR-Bs modRNA, leading to the dominance of Bn over Bs and global transcript degradation and therefore repressing reporter EGFP expression.

To identify the optimal ratio of nvUTR-Bs to sgUTR-Bn that produces the largest difference in reporter expression between Nsp1+ (with Nsp1) and Nsp1- (without Nsp1) states, we tested several different combinations of the two constituent modRNAs with the sgUTR-msfGFP reporter as a model of viral RNA expression. To observe how the system fares across different conditions, we tested three cell lines: the previously tested A549 and HuH7-ACE2 as well as HEK293FT. We observed a significant rise in the on-off reporter ratio when Nsp1 nvUTR-Bs/sgUTR-Bn is larger than 2 (Figures 2B–D, S3 and S4). In particular, a Bs/Bn ratio of 3 produced the largest difference in mean fluorescence intensity (MFI) between Nsp1+ and Nsp1- conditions in A549 cells (15.3-fold), while a ratio of 4 produced the largest differences in HEK293FT (24.0-fold) and HuH7-ACE2 (19.6-fold). Since the performance of the Bs/Bn ratio of 4 configuration in A549 is acceptable (12.9-fold), to minimize the amount of introduced barnase in the system, a Bs/Bn ratio of 4 was used for all subsequent experiments. To test the sensitivity of our system, we subjected the Bn-Bs system to decreasing amounts of Nsp1 mRNA (sgUTR-Nsp1), down to 3.125 ng per 1×10^5 cells at seeding (24-well plate). The system could detect Nsp1 even at the lowest sgUTR-Nsp1 dose tested, albeit at a lower Nsp1:Nsp1+ ratio (Figures 2D–F and S5). Altogether, we have designed a sensitive modRNA-based system capable of detecting SARS-CoV-2 Nsp1 and mediating global transcript degradation in its presence.

In this report, we demonstrated the construction of SARS-CoV-2 Nsp1-resistant modRNAs. We discovered the resistance-enhancing effects of m1 Ψ and m5C on sgUTR-containing modRNAs and the deleterious effects of pseudouridine on such modRNAs. An earlier report suggested the dampening and enhancing effects of Ψ and m1 Ψ , respectively, on certain RBP-responsive mRNA switches, which have been speculated to be caused by the alteration of sequence-specific affinity of RNA bases or RBPs.²⁴ However, while in the case of RBP-responsive mRNA switches, the enhancement was attributed to the stabilization of the secondary structures of RBP-binding aptamers; such a mechanism is unlikely to be the major contributor in the case of Nsp1-SL1 interaction since it

has been shown that SL1 mutations, which maintain the secondary structure, abolish the preference of viral RNA by Nsp1, suggesting the precedence of sequence over structure.^{17,25} While it is currently thought that Nsp1 is displaced by viral RNA during translation initiation, the exact mechanism of resistance of SL1-containing mRNA against Nsp1 remains elusive due to uncertainties surrounding the directness of the Nsp1-SL1 interaction and whether any interaction with host factors is required.^{16,23,26–31} Based on the current understanding of this phenomenon, we speculate that the combination of m1 Ψ and m5C outperforms other nucleoside combinations due to more stable interactions between Nsp1 and SL1 (and hypothetical host factors), resulting in a more efficient displacement of Nsp1 from its canonical ribosome-binding state, which allows sgUTR mRNA to be translated.

We also utilized such knowledge to design an Nsp1-sensing Bn-Bs system, which we optimized and demonstrated as capable of detecting even small amounts of SARS-CoV-2 Nsp1. Additionally, this system can potentially eliminate cells expressing Nsp1. Considering that the mechanism of selective recognition of viral RNA by Nsp1 is conserved in both the closely related SARS-CoV (Sarbecovirus) and the distantly related betacoronavirus MERS-CoV (Merbecovirus),^{33–36} it is likely that the same system is also applicable across different betacoronavirus subgenera. In addition, it has also been demonstrated that Nsp1-SL1 pairing with different sequence specificity can be generated by engineering paired mutations between the two components, opening the possibility of orthogonal pairings.³⁷ Taken together, these results indicate that our modRNA-based system is suitable for protein-responsive exclusive selector genetic circuits in general and SARS-CoV-2 protein-responsive genetic circuits in particular, which have potential implications for both the construction of more complex RNA-based genetic circuits and the development of modRNA therapeutics and tools for COVID-19 and other coronavirus disease research.

METHODS

Construction of Reporter Plasmid. pUC19 msfGFP was constructed as follows. The msfGFP CDS was amplified from pC034-LwCas13a-msfGFP-2A-Blast (Addgene plasmid # 91924; <http://n2t.net/addgene:91924>; RRID:Addgene_91924; gift from Zhang)³⁸ with PCR. Primers were designed with overhangs homologous to the linearized pUC19 backbone with modified 5'- and 3'-UTR sequences. The vector backbone and msfGFP CDS fragments were assembled by using the In-Fusion HD Cloning Kit (Takara Bio). Primer sequences used in the construction of pUC19 are shown in Table S1.

mRNA Synthesis. Templates for in vitro transcription (IVT) were prepared as described previously, with modifications.^{1,39} nvUTR 5'-UTR-containing IVT templates were generated by amplifying template plasmids using primers containing 5'-overhangs with the T7 RNA polymerase promoter sequence and poly-A tract (template already has 5'- and 3'-UTRs). sgUTR 5'-UTR-containing IVT templates, except for SARS-CoV-2 Nsp1, were generated by amplifying template plasmids with primers targeting the 5'-end of the CDS and the 3'-end of the 3'-UTR. The sgUTR 5'-UTR fragment was synthesized separately by PCR amplification. To generate the full template, the sgUTR 5'-UTR and CDS 3'-UTR fragments were fused by PCR with 5'-overhang primers as described above. For synthesizing the SARS-CoV-2 Nsp1

IVT template, a SARS-CoV-2 total genomic RNA isolate was reverse-transcribed with the SuperScript IV First-Strand Synthetic System (Thermo Fisher Scientific). The resultant cDNA was used as a template for PCR to amplify the coding sequence of Nsp1, which was used to generate the IVT template of sgUTR-Nsp1 mRNA as described above. Primer sequences and plasmids used for constructing IVT templates in this study are specified in Tables S2 and S3, respectively.

Synthetic mRNA synthesis was performed using a MEGA-Script T7 Transcription Kit (Thermo Fisher Scientific). nvUTR-barstar and sgUTR-barnase mRNAs were transcribed with 5-methylcytidine-5'-triphosphate (m5CTP) and N1-methylpseudouridine-5'-triphosphate (m1ΨTP) (TriLink BioTechnologies) in place of cytidine-5'-triphosphate (CTP) and uridine-5'-triphosphate, respectively. sgUTR-Nsp1 mRNA was synthesized with m5CTP in place of CTP and pseudouridine-5'-triphosphate (ΨTP) instead of m1ΨTP. nvUTR-GFP and sgUTR-GFP mRNAs were synthesized with either natural nucleotides (ATP, GTP, CTP, and UTP), modified nucleotides stated above in place of CTP and UTP, or combinations thereof. The transcription reaction mixture contained 7.5 mM ATP, 7.5 mM UTP, ΨTP, or m1ΨTP, 7.5 mM CTP or m5CTP, 1.5 mM GTP, and 6 mM Anti-Reverse Cap Analogue (TriLink BioTechnologies). The transcription reaction was performed by incubating the mixture at 37 °C for 6 h. After incubation, samples were treated with TURBO DNase (Thermo Fisher Scientific) and Antarctic Phosphatase (New England Biolabs) for 30 min at 37 °C. mRNA purification was performed with a Monarch RNA Cleanup Kit (50 μg) (New England Biolabs) according to manufacturer-provided protocol. The mRNA sequences used are specified in Table S4.

Cell Culture. A549 cells were cultured (37 °C, 5% CO₂) in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L glucose (Nacalai Tesque) supplemented with 10% fetal bovine serum (FBS) (Biosera). HEK293FT cells (Thermo Fisher Scientific) were cultured (37 °C, 5% CO₂) in DMEM with 4.5 g/L glucose (Nacalai Tesque) supplemented with 10% FBS (Biosera), 2 mM L-glutamine (Thermo Fisher Scientific), 1× MEM nonessential amino acids (Thermo Fisher Scientific), and 1 mM sodium pyruvate (Sigma). HuH-7-ACE2 cells were cultured (37 °C, 5% CO₂) in DMEM with 4.5 g/L glucose (Nacalai Tesque) supplemented with 10% FBS (Biosera) and 2 mM L-glutamine (Thermo Fisher Scientific).

For transfection, cells were trypsinized and plated into 24-, 48-, or 96-well plates at a density of 1×10^5 /mL (A549) or 2×10^5 /mL (HEK293FT and HuH-7-ACE2) at 24 h prior to transfection. Synthetic mRNA transfection was performed using Lipofectamine MessengerMAX (Thermo Fisher Scientific) according to the manufacturer-provided protocol.

Establishment of ACE2-Expressing Huh-7 Cells. Huh-7 cells (Cat #JCRB0403, JCRB Cell Bank) were electroporated with pPV-EF1α-ACE2-A and pHL-EF1α-hcPBα-A vectors⁴⁰ using a NEPA21 electroporator (Nepa Gene) and selected with 1 μg/mL puromycin (InvivoGen). The piggyBac-based ACE2-expressing plasmid, pPV-EF1α-ACE2-A, was constructed by replacing the EGFP CDS of pPV-EF1α-EiP-A with the ACE2 CDS. pPV-EF1α-EiP-A is a piggyBac-based EGFP-expressing plasmid.

Flow Cytometry. Twenty-four h after transfection, cells were washed with phosphate-buffered saline (PBS). Cells were detached using 50 μL of 0.25% Trypsin-EDTA (Thermo Fisher Scientific) at 37 °C for 5 min. After incubation, 150 μL of fresh medium was added. Cells were passed through a nylon

mesh and analyzed using a BD Accuri C6 flow cytometer (BD Biosciences) (A549) or a CytoFLEX S flow cytometer (HEK293FT and HuH-7-ACE2). For the BD Accuri C6, the GFP signal was detected by an FL1 (533/30 nm) filter with 90% attenuation, and the iRFP670 signal was detected by an FL4 (675/25 nm) filter. For the CytoFLEX S, the GFP signal was excited with a 488 nm laser and detected by a 525/40 nm filter, and the iRFP670 signal was excited with a 638 nm laser and detected by a 660/10 nm filter.

Obtained flow cytometry data (in FCS format) were analyzed using FlowJo ver. 10.6.1 (BD Biosciences) and R. For all analyses, gates were generated with mock samples. Debris elimination (P1 gate) was performed using forward vs side scatter (FSC-A against SSC-A). For dual-fluorescence analysis, the iRFP670-positive (iRFP670⁺) population was defined as cells with iRFP670 fluorescence intensity values exceeding the 99.9% of the mock population. Relative fluorescence intensity was obtained by dividing the GFP intensity value by the iRFP670 intensity value for each cell. For single-fluorescence analysis, the GFP-positive (GFP⁺) population was obtained by marking the population with GFP intensity higher than 99% of the mock population in the GFP versus SSC-A scatter plot.

Statistical Analysis. All bar charts and error bars represent mean values and standard deviations (SD) from three independent biological replicates, respectively. All statistical analyses were performed using an unpaired, two-tailed Student's *t*-test using R. Significance levels: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, and not significant (*P* ≥ 0.05).

■ ASSOCIATED CONTENT

Data Availability Statement

All raw data and experimental materials are available upon reasonable request to the corresponding author.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssynbio.5c00075>.

Additional flow cytometry and cell imaging data and details of materials used in this study, raw fluorescence intensity and fluorescence-positive percentage data; representative contour plots and histograms; representative scatter plots; representative cell images; representative scatter plots and cell images of sensitivity experiment; primers used in plasmid construction; primers used in IVT mRNA template construction; plasmids used in this study; and mRNA sequences used in this study (PDF)

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Author Contributions

M.L.P. and H.S. performed biochemical and reporter experiments. K.T. established the Huh7-ACE2 cell line. H.S. supervised the project. M.L.P., K.T., and H.S. wrote the manuscript with contributions from all authors.

Notes

The authors declare the following competing financial interest(s): H.S. is the investigator of the patent application related to RNA switch technologies broadly relevant to this work. H.S. own shares of aceRNA Technologies Ltd. and is an external director of aceRNA Technologies Ltd. Other authors declare no competing interests.

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REFERENCES

- (1) Wroblewska, L.; Kitada, T.; Endo, K.; Siciliano, V.; Stillo, B.; Saito, H.; Weiss, R. Mammalian Synthetic Circuits with RNA Binding Proteins Delivered by RNA. *Nat. Biotechnol.* **2015**, *33* (8), 839–841.
- (2) Pardi, M. L.; Wu, J.; Kawasaki, S.; Saito, H. Synthetic RNA-Based Post-Transcriptional Expression Control Methods and Genetic Circuits. *Adv. Drug Delivery Rev.* **2022**, *184*, No. 114196.
- (3) Sahin, U.; Karikó, K.; Türeci, Ö. mRNA-Based Therapeutics—Developing a New Class of Drugs. *Nat. Rev. Drug Discovery* **2014**, *13* (10), 759–780.
- (4) Pfeifer, B. A.; Beitelshees, M.; Hill, A.; Bassett, J.; Jones, C. H. Harnessing Synthetic Biology for Advancing RNA Therapeutics and Vaccine Design. *Npj Syst. Biol. Appl.* **2023**, *9* (1), 1–10.
- (5) Kawasaki, S.; Fujita, Y.; Nagaike, T.; Tomita, K.; Saito, H. Synthetic mRNA Devices That Detect Endogenous Proteins and Distinguish Mammalian Cells. *Nucleic Acids Res.* **2017**, *45* (12), No. e117.
- (6) Kawasaki, S.; Ono, H.; Hirosawa, M.; Kuwabara, T.; Sumi, S.; Lee, S.; Woltjen, K.; Saito, H. Programmable Mammalian Translational Modulators by CRISPR-Associated Proteins. *Nat. Commun.* **2023**, *14*, 2243.
- (7) Saito, H.; Kobayashi, T.; Hara, T.; Fujita, Y.; Hayashi, K.; Furushima, R.; Inoue, T. Synthetic Translational Regulation by an L7Ae–Kink-Turn RNP Switch. *Nat. Chem. Biol.* **2010**, *6* (1), 71–78.
- (8) DiAndreth, B.; Wauford, N.; Hu, E.; Palacios, S.; Weiss, R. PERSIST Platform Provides Programmable RNA Regulation Using CRISPR endoRNases. *Nat. Commun.* **2022**, *13* (1), 2582.
- (9) Matsuura, S.; Ono, H.; Kawasaki, S.; Kuang, Y.; Fujita, Y.; Saito, H. Synthetic RNA-Based Logic Computation in Mammalian Cells. *Nat. Commun.* **2018**, *9* (1), 4847.
- (10) Ono, H.; Kawasaki, S.; Saito, H. Orthogonal Protein-Responsive mRNA Switches for Mammalian Synthetic Biology. *ACS Synth. Biol.* **2020**, *9* (1), 169–174.
- (11) Nakanishi, H.; Saito, H. Caliciviral Protein-Based Artificial Translational Activator for Mammalian Gene Circuits with RNA-Only Delivery. *Nat. Commun.* **2020**, *11* (1), 1297.
- (12) Nakanishi, H.; Yoshii, T.; Kawasaki, S.; Hayashi, K.; Tsutsui, K.; Oki, C.; Tsukiji, S.; Saito, H. Light-Controllable RNA-Protein Devices for Translational Regulation of Synthetic mRNAs in Mammalian Cells. *Cell Chem. Biol.* **2021**, *28* (5), 662–674.e5.
- (13) Finkel, Y.; Gluck, A.; Nachshon, A.; Winkler, R.; Fisher, T.; Rozman, B.; Mizrahi, O.; Lubelsky, Y.; Zuckerman, B.; Slobodin, B.; Yahalom-Ronen, Y.; Tamir, H.; Ulitsky, I.; Israely, T.; Paran, N.; Schwartz, M.; Stern-Ginossar, N. SARS-CoV-2 Uses a Multipronged Strategy to Impede Host Protein Synthesis. *Nature* **2021**, *594*, 240–245.
- (14) Schubert, K.; Karousis, E. D.; Jomaa, A.; Scaiola, A.; Echeverria, B.; Gurzeler, L.-A.; Leibundgut, M.; Thiel, V.; Mühlemann, O.; Ban, N. SARS-CoV-2 Nsp1 Binds the Ribosomal mRNA Channel to Inhibit Translation. *Nat. Struct. Mol. Biol.* **2020**, *27* (10), 959–966.
- (15) Thoms, M.; Buschauer, R.; Ameisemeier, M.; Koepke, L.; Denk, T.; Hirschenberger, M.; Kratzat, H.; Hayn, M.; Mackens-Kiani, T.; Cheng, J.; Straub, J. H.; Stürzel, C. M.; Fröhlich, T.; Berninghausen, O.; Becker, T.; Kirchhoff, F.; Sparrer, K. M. J.; Beckmann, R. Structural Basis for Translational Shutdown and Immune Evasion by the Nsp1 Protein of SARS-CoV-2. *Science* **2020**, *369* (6508), 1249–1255.
- (16) Tidu, A.; Janvier, A.; Schaeffer, L.; Sosnowski, P.; Kuhn, L.; Hammann, P.; Westhof, E.; Eriani, G.; Martin, F. The Viral Protein NSP1 Acts as a Ribosome Gatekeeper for Shutting down Host Translation and Fostering SARS-CoV-2 Translation. *RNA* **2021**, *27* (3), 253–264.
- (17) Bujanic, L.; Shevchuk, O.; von Kügelgen, N.; Kalina, A.; Ludwik, K.; Koppstein, D.; Zerna, N.; Sickmann, A.; Chekulaeva, M. The Key Features of SARS-CoV-2 Leader and NSP1 Required for Viral Escape of NSP1-Mediated Repression. *RNA* **2022**, *28* (5), 766–779.
- (18) Tardivat, Y.; Sosnowski, P.; Tidu, A.; Westhof, E.; Eriani, G.; Martin, F. SARS-CoV-2 NSP1 Induces mRNA Cleavages on the Ribosome. *Nucleic Acids Res.* **2023**, *51*, 8677–8690.
- (19) Anderson, B. R.; Muramatsu, H.; Nallagatla, S. R.; Bevilacqua, P. C.; Sansing, L. H.; Weissman, D.; Karikó, K. Incorporation of Pseudouridine into mRNA Enhances Translation by Diminishing PKR Activation. *Nucleic Acids Res.* **2010**, *38* (17), 5884–5892.
- (20) Karikó, K.; Muramatsu, H.; Welsh, F. A.; Ludwig, J.; Kato, H.; Akira, S.; Weissman, D. Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability. *Mol. Ther. J. Am. Soc. Gene Ther.* **2008**, *16* (11), 1833–1840.
- (21) Nance, K. D.; Meier, J. L. Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. *ACS Cent. Sci.* **2021**, *7* (5), 748–756.
- (22) Sasaki, M.; Kishimoto, M.; Itakura, Y.; Tabata, K.; Intaruck, K.; Uemura, K.; Toba, S.; Sanaki, T.; Sato, A.; Hall, W. W.; Orba, Y.; Sawa, H. Air-Liquid Interphase Culture Confers SARS-CoV-2 Susceptibility to A549 Alveolar Epithelial Cells. *Biochem. Biophys. Res. Commun.* **2021**, *577*, 146–151.
- (23) Vora, S. M.; Fontana, P.; Mao, T.; Leger, V.; Zhang, Y.; Fu, T.-M.; Lieberman, J.; Gehrke, L.; Shi, M.; Wang, L.; Iwasaki, A.; Wu, H. Targeting Stem-Loop 1 of the SARS-CoV-2 5′ UTR to Suppress Viral Translation and Nsp1 Evasion. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (9), No. e2117198119.
- (24) Parr, C. J. C.; Wada, S.; Kotake, K.; Kameda, S.; Matsuura, S.; Sakashita, S.; Park, S.; Sugiyama, H.; Kuang, Y.; Saito, H. N1-Methylpseudouridine Substitution Enhances the Performance of Synthetic mRNA Switches in Cells. *Nucleic Acids Res.* **2020**, *48* (6), No. e35.
- (25) Slobodin, B.; Sehwat, U.; Lev, A.; Hayat, D.; Zuckerman, B.; Fraticelli, D.; Ogran, A.; Ben-Shmuel, A.; Bar-David, E.; Levy, H.; Ulitsky, I.; Dikstein, R. Cap-Independent Translation and a Precisely Located RNA Sequence Enable SARS-CoV-2 to Control Host

Translation and Escape Anti-Viral Response. *Nucleic Acids Res.* **2022**, 50 (14), 8080–8092.

(26) Wang, Y.; Kirkpatrick, J.; Zur Lage, S.; Carlomagno, T. Structural Insights into the Activity Regulation of Full-Length Non-Structural Protein 1 from SARS-CoV-2. *Structure* **2023**, 31 (2), 128–137.e5.

(27) Shi, M.; Wang, L.; Fontana, P.; Vora, S.; Zhang, Y.; Fu, T.-M.; Lieberman, J.; Wu, H. SARS-CoV-2 Nsp1 Suppresses Host but Not Viral Translation through a Bipartite Mechanism. *bioRxiv* 2020 .

(28) Vankadari, N.; Jeyasankar, N. N.; Lopes, W. J. Structure of the SARS-CoV-2 Nsp1/5'-Untranslated Region Complex and Implications for Potential Therapeutic Targets, a Vaccine, and Virulence. *J. Phys. Chem. Lett.* **2020**, 11 (22), 9659–9668.

(29) Lapointe, C. P.; Grosely, R.; Johnson, A. G.; Wang, J.; Fernández, I. S.; Puglisi, J. D. Dynamic Competition between SARS-CoV-2 NSP1 and mRNA on the Human Ribosome Inhibits Translation Initiation. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, 118 (6), No. e2017715118.

(30) Aviner, R.; Lidsky, P. V.; Xiao, Y.; Tassetto, M.; Kim, D.; Zhang, L.; McAlpine, P. L.; Elias, J.; Frydman, J.; Andino, R. SARS-CoV-2 Nsp1 Cooperates with Initiation Factors EIF1 and 1A to Selectively Enhance Translation of Viral RNA. *PLOS Pathog.* **2024**, 20 (2), No. e1011535.

(31) Schubert, K.; Karousis, E. D.; Ban, I.; Lapointe, C. P.; Leibundgut, M.; Bäuml, E.; Kummerant, E.; Scaiola, A.; Schönhut, T.; Ziegelmüller, J.; Puglisi, J. D.; Mühlemann, O.; Ban, N. Universal Features of Nsp1-Mediated Translational Shutdown by Coronaviruses. *Mol. Cell* **2023**, 83 (19), 3546–3557.e8.

(32) Fujita, Y.; Hirose, M.; Hayashi, K.; Hatani, T.; Yoshida, Y.; Yamamoto, T.; Saito, H. A Versatile and Robust Cell Purification System with an RNA-Only Circuit Composed of microRNA-Responsive ON and OFF Switches. *Sci. Adv.* **2022**, 8 (1), No. eabj1793.

(33) Huang, C.; Lokugamage, K. G.; Rozovics, J. M.; Narayanan, K.; Semler, B. L.; Makino, S. SARS Coronavirus Nsp1 Protein Induces Template-Dependent Endonucleolytic Cleavage of mRNAs: Viral mRNAs Are Resistant to Nsp1-Induced RNA Cleavage. *PLOS Pathog.* **2011**, 7 (12), No. e1002433.

(34) Terada, Y.; Kawachi, K.; Matsuura, Y.; Kamitani, W. MERS Coronavirus Nsp1 Participates in an Efficient Propagation through a Specific Interaction with Viral RNA. *Virology* **2017**, 511, 95–105.

(35) Devarkar, S. C.; Vetic, M.; Balaji, S.; Lomakin, I. B.; Yang, L.; Jin, D.; Gilbert, W. V.; Chen, S.; Xiong, Y. Structural Basis for Translation Inhibition by MERS-CoV Nsp1 Reveals a Conserved Mechanism for Betacoronaviruses. *Cell Rep.* **2023**, 42 (10), No. 113156.

(36) Maurina, S. F.; O'Sullivan, J. P.; Sharma, G.; Pineda Rodriguez, D. C.; MacFadden, A.; Cendali, F.; Henen, M. A.; Vögeli, B.; Kieft, J. S.; Glasgow, A.; Steckelberg, A.-L. An Evolutionarily Conserved Strategy for Ribosome Binding and Host Translation Inhibition by β -Coronavirus Non-Structural Protein 1. *J. Mol. Biol.* **2023**, 435 (20), No. 168259.

(37) Sosnowski, P.; Tidu, A.; Eriani, G.; Westhof, E.; Martin, F. Correlated Sequence Signatures Are Present within the Genomic 5'UTR RNA and NSP1 Protein in Coronaviruses. *RNA* **2022**, 28 (5), 729–741.

(38) Abudayyeh, O. O.; Gootenberg, J. S.; Essletzbichler, P.; Han, S.; Joung, J.; Belanto, J. J.; Verdine, V.; Cox, D. B. T.; Kellner, M. J.; Regev, A.; Lander, E. S.; Voytas, D. F.; Ting, A. Y.; Zhang, F. RNA Targeting with CRISPR–Cas13. *Nature* **2017**, 550 (7675), 280–284.

(39) Miki, K.; Endo, K.; Takahashi, S.; Funakoshi, S.; Takei, I.; Katayama, S.; Toyoda, T.; Kotaka, M.; Takaki, T.; Umeda, M.; Okubo, C.; Nishikawa, M.; Oishi, A.; Narita, M.; Miyashita, I.; Asano, K.; Hayashi, K.; Osafune, K.; Yamanaka, S.; Saito, H.; Yoshida, Y. Efficient Detection and Purification of Cell Populations Using Synthetic MicroRNA Switches. *Cell Stem Cell* **2015**, 16 (6), 699–711.

(40) Ishida, K.; Xu, H.; Sasakawa, N.; Lung, M. S. Y.; Kudryashev, J. A.; Gee, P.; Hotta, A. Site-Specific Randomization of the Endogenous

Genome by a Regulatable CRISPR-Cas9 piggyBac System in Human Cells. *Sci. Rep.* **2018**, 8 (1), 310.

Supporting Information

SARS-CoV-2 Nsp1-resistant modified RNA for the creation of Nsp1-responsive systems

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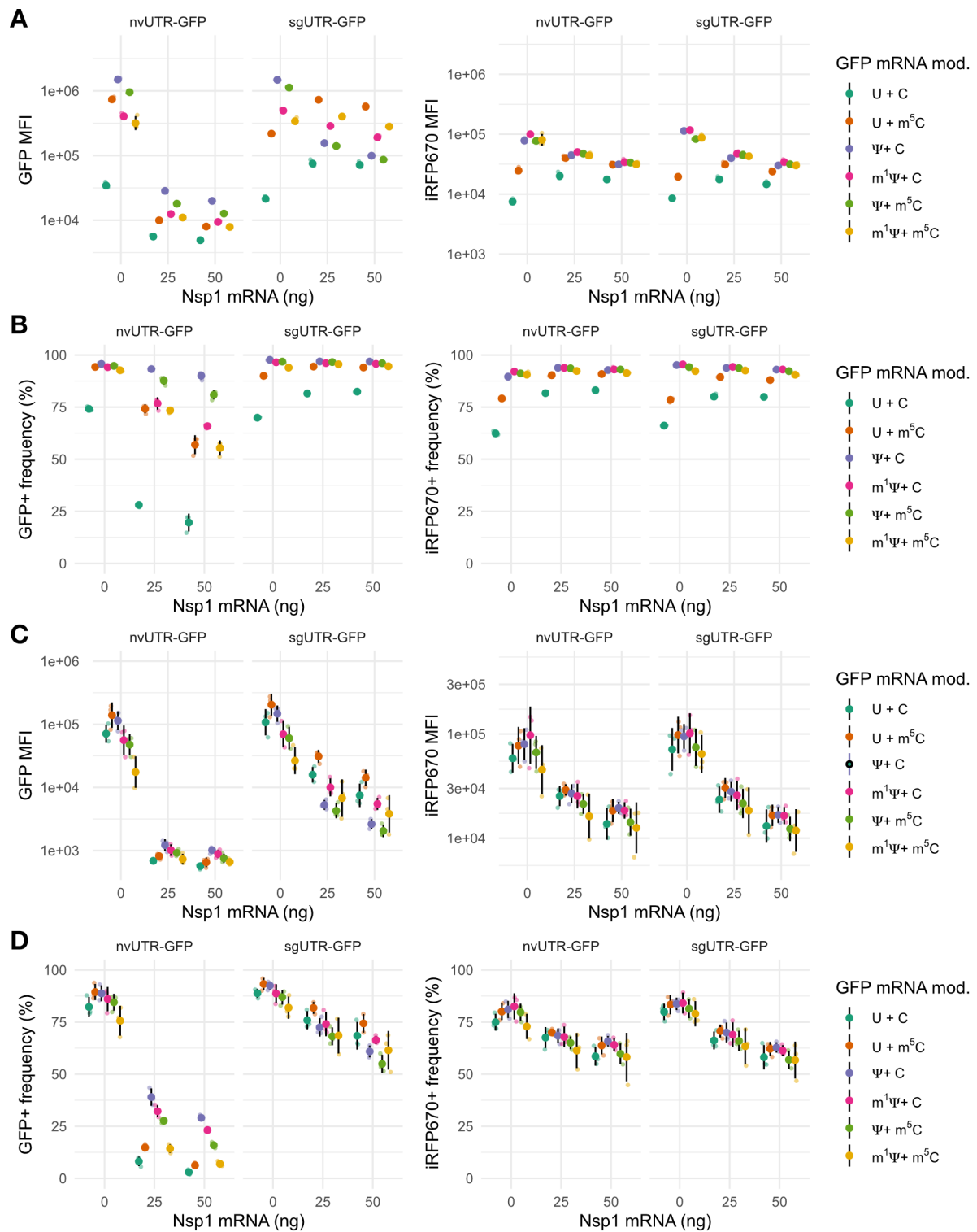


Figure S1. Raw fluorescence intensity and fluorescence-positive percentage data, related to Figure 1

(A and B) GFP and iRFP670 fluorescence intensity data (A) and GFP- and iRFP670-positive percentage data (B) derived from nvUTR/sgUTR-GFP and sgUTR-iRFP670 modRNA, with or without Nsp1, in A549 cells. (C and D) GFP and iRFP670 fluorescence intensity data (C) and GFP- and iRFP670-positive percentage data (D) derived from nvUTR/sgUTR-GFP and sgUTR-iRFP670 modRNA, with or without Nsp1, in and HuH-7-ACE2 (D) cells. Smaller, transparent dots represent individual replicate data points, while larger, opaque dots represent mean values of three biological replicates.

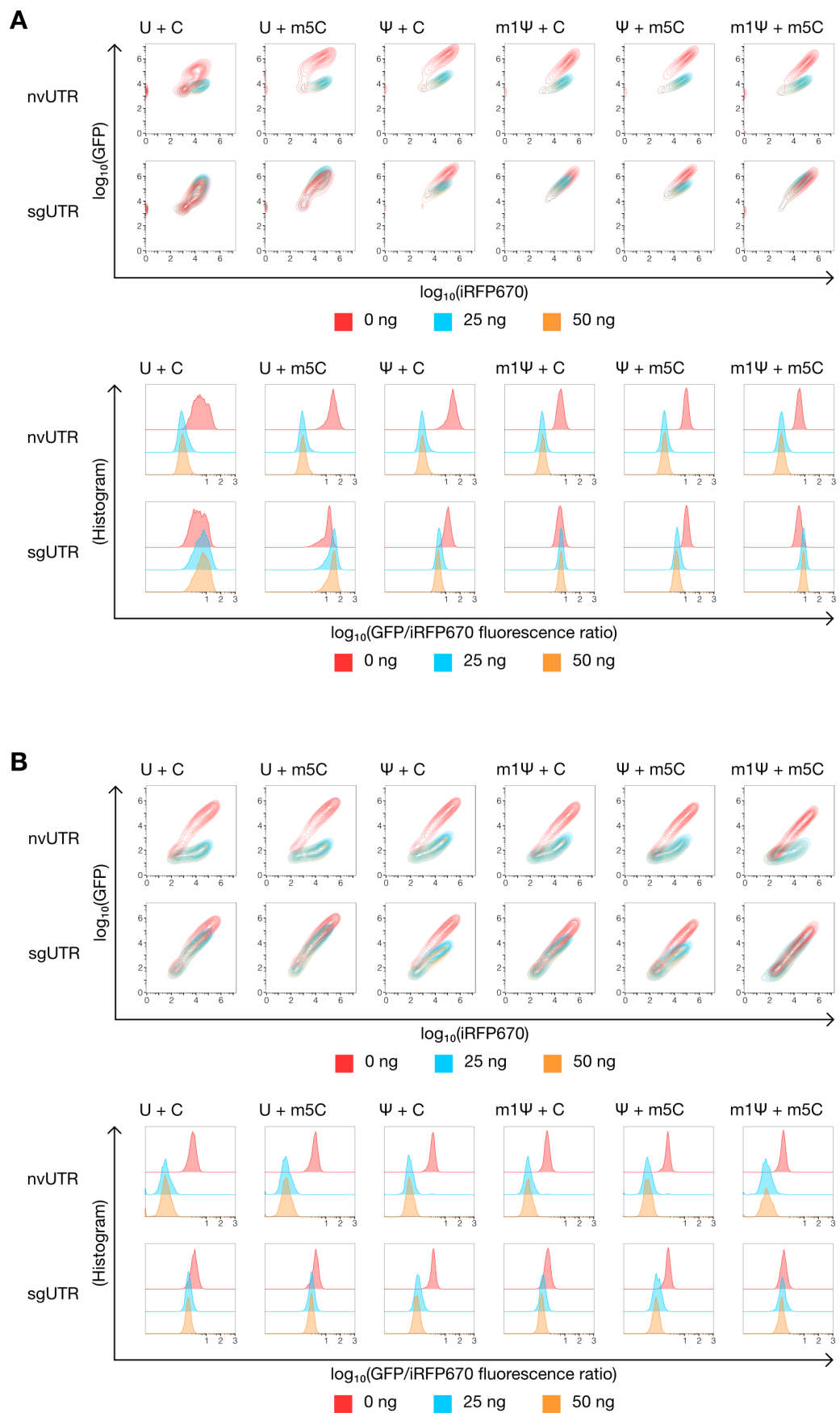


Figure S2. Representative contour plots and histograms related to Figure 1

(A and B) Representative data of fluorescence intensity derived from nvUTR/sgUTR-GFP and sgUTR-iRFP670 modRNA, with or without Nsp1, in A549 (A) and HuH-7-ACE2 (B) cells. Red: cells co-transfected with nvUTR/sgUTR-GFP and sgUTR-iRFP670 at a 1:1 mass ratio. Blue: cells co-transfected with nvUTR/sgUTR-GFP, sgUTR-iRFP670, and sgUTR-Nsp1 at a 1:1:0.5 mass ratio. Orange: cells co-transfected with nvUTR/sgUTR-GFP, sgUTR-iRFP670, and sgUTR-Nsp1 at a 1:1:1 mass ratio.

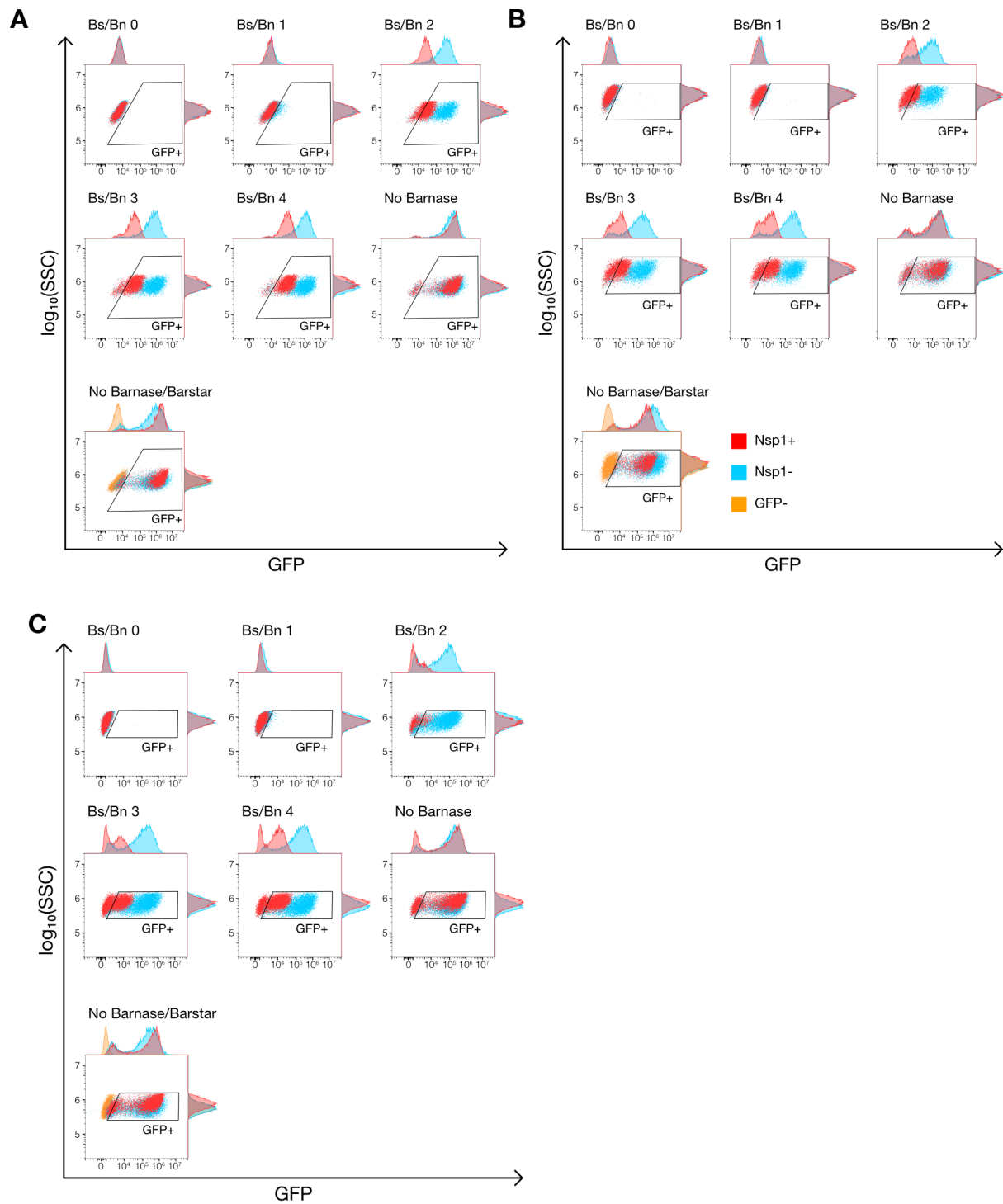


Figure S3. Representative scatter plots related to Figure 2

(A-C) Representative data of fluorescence intensity derived from sgUTR-GFP under various nvUTR-barstar/sgUTR-barnase combinations, with or without Nsp1, in A549 (A), HuH-7-ACE2 (B), and HEK293FT (C) cells. Red: cells co-transfected with sgUTR-GFP and nvUTR-barstar/sgUTR-barnase at the indicated ratio (Bs/Bn), without sgUTR-Nsp1. Blue: cells co-transfected with sgUTR-GFP, nvUTR-barstar/sgUTR-barnase at the indicated ratio (Bs/Bn), with sgUTR-Nsp1. Orange: cells transfected with sgUTR-GFP only.

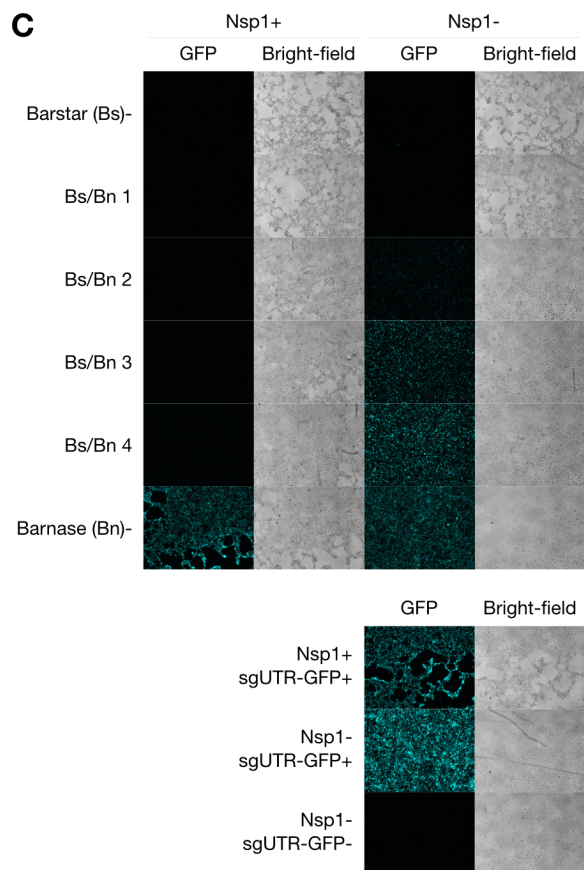
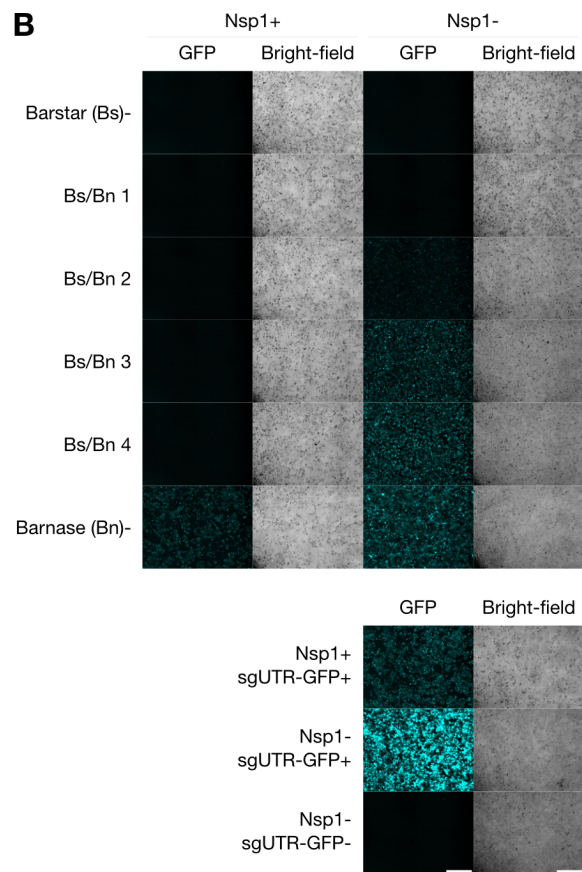
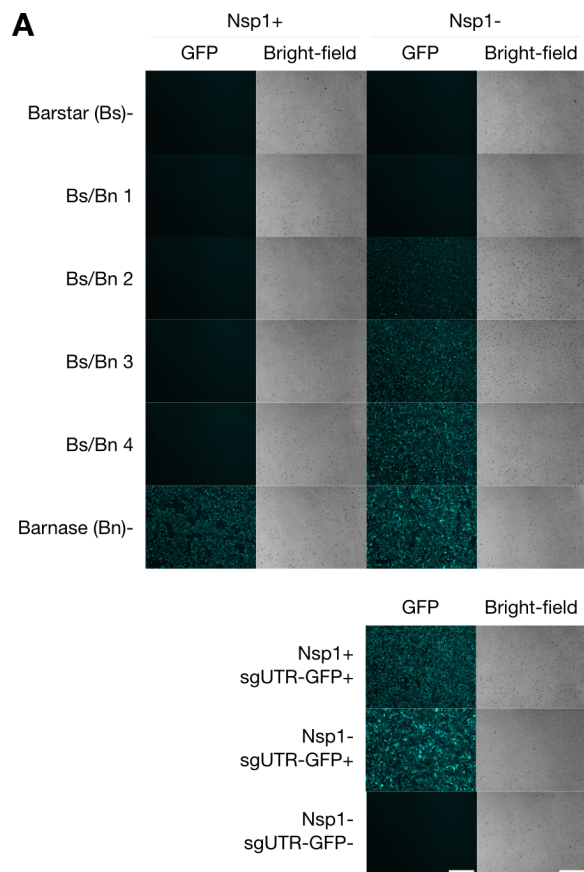


Figure S4. Representative cell images related to Figure 2

(A-C) Representative GFP fluorescence and bright-field images of cells transfected with sgUTR-GFP and nvUTR-barstar/sgUTR-barnase mixture under the indicated ratio in A549 (A), HuH-7-ACE2 (B), and HEK293FT (C) cells. Scale bar: 500 μ m

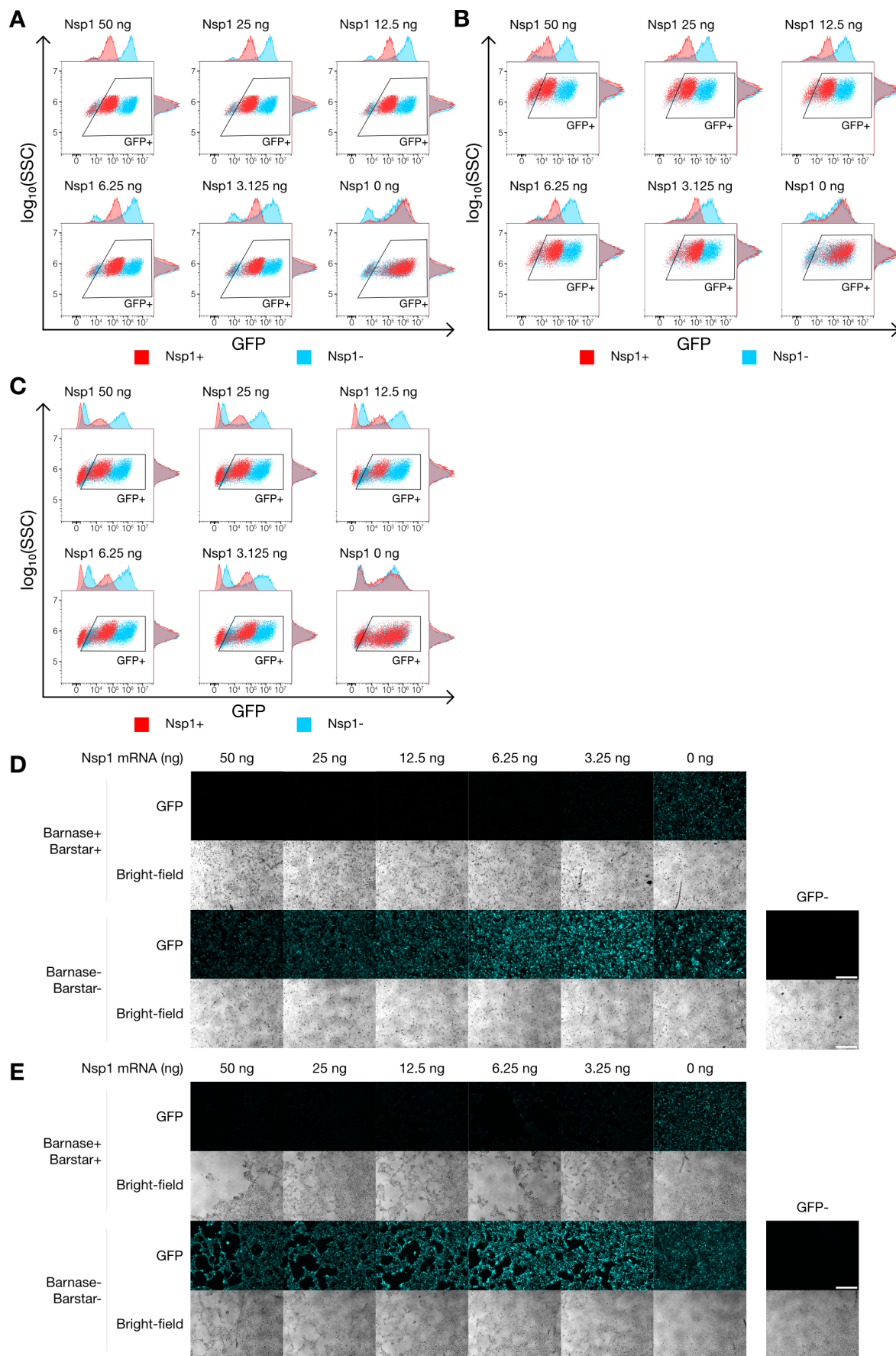


Figure S5. Representative scatter plots and cell images of sensitivity experiment related to Figure 2

(A-C) Representative data of fluorescence intensity derived from sgUTR-GFP co-transfected with nvUTR-barstar/sgUTR-barnase 4:1 mixture and various amounts of Nsp1 in A549 (A), HuH-7-ACE2 (B), and HEK293FT (C) cells. Red: cells co-transfected with sgUTR-GFP and nvUTR-barstar/sgUTR-barnase with sgUTR-Nsp1 at the indicated. Blue: cells co-transfected with sgUTR-GFP, nvUTR-barstar/sgUTR-barnase with sgUTR-Nsp1. (D and E) Representative GFP fluorescence and bright-field images of cells transfected with sgUTR-GFP and nvUTR-barstar/sgUTR-barnase 4:1 mixture under various amounts of Nsp1 in HuH-7-ACE2 (D) and HEK293FT (E) cells. Scale bar: 500 μm

Table S1. Primers used in plasmid construction

Name	Sequence
MLP-043 msfGFP ORF FWD	CACCGGTCGCCACCATGGTGAGTAAAGGTGAAGAACTCTT CACTGG
MLP-044 msfGFP ORF REV	GCCCCGCAGAAGGTCTTACTTGTACAGCTCATCCATACCG AGA

Table S2. Primers used in IVT mRNA template construction

Name	Sequence
MLP-182 sgRNA-N	CAGTGAATTGTAATACGACTCACTATAGATTAAAGGTTTATACCTTCCCA GGTAACAAACCAACCAACTTTTCGATCTCTTGTAGATCTGTTCTCTAAACG AACAAACTAAA
MLP-183 sgRNA_5UTR SCoV2 FWD	CAGTGAATTGTAATACGACTCACTATAGATTAAAGG
MLP-185 sg_5UTR SCoV2 REV Nsp1	CCAGGGACAAGGCTCTCCATTTTAGTTTGTTTCGTTTAGAGAACAGATCTA
MLP-223 sg_5UTR SCoV2 REV msfGFP	AGTGAAGAGTTCTTCACCTTTACTCACCATTTTAGTTTGTTTCGTTTAGAGA ACAGATCTA
MLP-224 sg_5UTR SCoV2 REV iRFP	GAGATCGACCTTACGCGCCATTTTAGTTTGTTTCGTTTAGAGAACAGATCT A
MLP-405 sg_5UTR REV Barnase	GTGTTGATAACCTGTGCGGATCCCATTTTAGTTTGTTTCGTTTAGAGAACA GATCTA
MLP-186 SCoV2 Nsp1 FWD	ATGGAGAGCCTTGTCCTGG
MLP-187 SCoV2 Nsp1 REV	GCCCCGCAGAAGGTCTAGACCCTCCGTTAAGCTCACGC
MLP-231 msfGFP ORF FWD	ATGGTGAGTAAAGGTGAAGAACTCTTCACT
MLP-211 iRFP670- BF FWD	ATGGCGCGTAAGGTCGAT
MLP-408 Barnase ORF FWD	ATGGGATCCGCACAGGTTATCAACA
KEC-63 IVT_5prime_UTR	TCTAGACCTTCTGCGGGGCTTGCTTCTGGCCATGCCCTTCTTCTCTCC CTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGG
KEC-4 Fwd3UTR	TCTAGACCTTCTGCGGGGC
KEC-65 Rev3UTR2T20	TTTTTTTTTTTTTTTTTTTCTACTCAGGCTTTATTCAAAGACCAAG
YF771 T7_5UTR- fwd	ATTGTAATACGACTCACTATAGGGCGAATTAAGAGAGAAAAGAAGAGTA AG
KED-88 3UTRT120	(T120)CCTACTCAGGCTTTATTCA

Table S3. Plasmids used in this study

Plasmid	Reference
pUC19-msfGFP	This paper
pUC19-iRFP670	Fujita et al., 2022 ¹
pUC19-Barstar	Fujita et al., 2022 ¹
pUC19-Barstar-UTR-Barnase	Fujita et al., 2022 ¹

Table S4. mRNA sequences used in this study

mRNA	Sequence
nvUTR- msfGFP	GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGC CACCAUGGUGAGUAAAGGUGAAGAACUCUUCACUGGAGUAGUGCCCAUUCU GGUAGAGCUUGAUGGAGAUGUAAAUGGACAUAAAUUCUCCGUCAGGGGCGA AGGCGAAGGGGACGCCACGAAUGGUAAAGCUGACUCUGAAAUUCAUCUGUAC GACGGGCAAACUGCCCCGUCCCAUGGCCUACACUCGUAACGACCCUCACCUAC GGCGUGCAAUGCUUUUCUGAUAUCCCGACCACAUGAACAGCAUGACUUUU UCAAGUCUGCAAUGCCUGAAGGUUAUGUUAAGAAAGGACCAUCAGCUUUAA GGAUGAUGGUACAUUAAAACCCGAGCCGAGGUUAAAUUUGAAGGGGACAC UCUGGUUAAUCGAAUUGAACUGAAAGGUUAUUGAUUUUAAAGGAGGACGGUAA CAUACUGGGGCACAAGUUGGAGUACAACUUUAACAGCCAUAUUGUUAUUAU ACCGCUGAUAAAGCAGAAAAAUGGGAUAAAGGCCAACUUUAAGAUCGACAUA AUGUCGAAGAUGGUAGUGUUAACUGGCGUAUCAUUAACCAACAAAAUACGCC CAUCGGAGAUGGACCUGUACUCUUGCCUGACAAUCAUUAUCUCUCCACGCAA UCAAAGCUUCCAAGGACCCAAACGAAAAGAGAGACAUCAGUCCUUCUGG AAUUUGUACUGCCGCGAGGCAUCACUCUGGUUAUGGAUGAGCUGUACAAGU AAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGC ACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAA AAA AAA
sgUTR- msfGFP	GAUUAAGGUUUUAUACCUUCCCAGGUAAACAAACCAACCUUUCGAUCUCUU GUAGAUCUGUUCUCUAAACGAACAAACUAAAAUGGUGAGUAAAGGUGAAGAAC UCUUCACUGGAGUAGUGCCCAUUCUGGUAGAGCUUGAUGGAGAUGUAAAUG GACAUAAAUUCUCCGUCAGGGGCGAAGGCGAAGGGGACGCCACGAAUGGUA AGCUGACUCUGAAAUUCAUCUGUACGACGGGCAAACUGCCCCGUCCCAUGGCC UACACUCGUAACGACCCUCACCUACGGCGUGCAAUGCUUUUCUGAUAUCCC GACCACAUGAAACAGCAUGACUUUUUCAAGUCUGCAAUGCCUGAAGGUUAUG UUAAGAAAGGACCAUCAGCUUUAAGGAUGAUGGUACAUAUAAAACCCGAGC CGAGGUUAAAUUUGAAGGGGACACUCUGGUUAAUCGAAUUGAACUGAAAGG UAUUGAUUUUUAAGGAGGACGGUAACAUAACUGGGGCACAAGUUGGAGUACAA CUUUAACAGCCAUAUUGUGUAUUAUACCGCUGAUAAAGCAGAAAAAUGGGUA AAGGCCAACUUUAAGAUCGACAUAAUGUCGAAGAUGGUAGUGUUAACUG GCUGAUCAUUAACCAACAAAAUACGCCCAUCGGAGAUGGACCUGUACUCUUGC CUGACAAUCAUUAUCUCUCCACGCAAUCAAAGCUUCCAAGGACCCAAACGA AAAGAGAGAUACAUGGUCCUUCUGGAAUUUGUGACUGCCGCGAGGCAUCAC UCUCGGUAUGGAUGAGCUGUACAAGUAAGACCUUCUGCGGGGCUUGCCUUC UGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUA AAGCCUGAGUAGGAAA AAA AAAAAAAAAAAAAAAAAAAAAAAAA

sgUTR- iRFP670	GAUUAAGGUUUAUACCUUCCAGGUAACAAACCAACCAACUUUCGAUCUCUU GUAGAUCUGUUCUCUAAACGAACAAACUAAAAUGGCGCGUAAGGUCGAUCUCA CCUCCUGCGAUCGCGAGCCGAUCCACAUCGCCGCGAGCAUUCAGCCGUGCG GCUGCCUGCUAGCCUGCGACGCGCAGGCGGUGCGGAUCACGCGCAUUACGG AAAAUGCCGCGCGUUCUUUGGACGCGAAACUCCGCGGGUCGGUGAGCUAC UCGCCGAUUACUUCGGCGAGACCGAAGCCCAUGCGCUGCGCAACGCACUGG CGCAGUCCUCCGAUCCAAAGCGACCGGCGCUGAUCUUCGGUUGGCGCGACG GCCUGACCGGCCGCACCUUCGACAUCUCACUGCAUCGCCAUGACGGUACAUC GAUCAUCGAGUUCGAGCCUGCGGCGGCCGAACAGGCCGACAAUCCGUGCG GCUGACGCGGCAGAUCAUCGCGCGCACCAAGAACUGAAGUCGCUCGAAGA GAUGGCCGCACGGGUGCCGCGCUAUCUGCAGGCGAUGCUCGGCUAUCACCG CGUGAUGUUGUACCGCUUCGCGGACGACGGCUCGCGGAUGGUGAUCGGCGA GGCGAAGCGCAGCGACCUCGAGAGCUUUCUGGUCAGCACUUUCCGGCGUC GCUGGUCCCGCAGCAGGCGCGGCUACUGUACUUGAAGAACGCGAUCCGCGU GGUCUCGGAUUCGCGCGGCAUCAGCAGCCGGAUCGUGCCCGAGCACGACGC CUCCGGCGCCGCGCUCGAUCUGUCGUUCGCGCACCUCCGCGCAGCAUCUCGCC CUGCCAUCUCGAAUUUCUGCGGAACAUGGGCGUCAGCGCCUCGAUGUCGU GUCGAUCAUCAUUGACGGCACGCUAUGGGGAUUGAUCAUCUGUCAUCAUAC GAGCCGCGUGCCGUGCCGAUGGCGCAGCGCGUCGCGGCCGAAAUGUUCGCC GACUUCUUAUCGUCGACUUCACCGCCGCCACCACCAACGCAGAUUCUAUA UGCAUCUCGAGUGAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU UCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAA AA AA AAA
sgUTR- Nsp1	GAUUAAGGUUUAUACCUUCCAGGUAACAAACCAACCAACUUUCGAUCUCUU GUAGAUCUGUUCUCUAAACGAACAAACUAAAAUGGAGAGCCUUGUCCUGGUU UCAACGAGAAAACACACGUCCAACUCAGUUUGCCUGUUUUACAGGUUCGCGA CGUGCUCGUACGUGGCUUUGGAGACUCCGUGGAGGAGGUCUUAUCAGAGGC ACGUCAACAUCUUAAGAUGGCACUUGUGGCUUAGUAGAAGUUGAAAAAGG CGUUUUGCCUCAACUUGAACAGCCCUAUGUGUUCAUCAAACGUUCGGAUGCU CGAACUGCACCUCUUGGUCAUGUUAUGGUUGAGCUGGUAGCAGAACUCGAA GGCAUUCAGUACGGUCGUAGUGGUGAGACACUUGGUGUCCUUGUCCCUCAU GUGGGCGAAAUACCAGUGGCUUACCGCAAGGUUCUUCUUCGUAAGAACGGU AAUAAAGGAGCUGGUGGCCAUAGUUACGGCGCCGAUCUAAAGUCAUUUGAC UUAGGCGACGAGCUUGGCACUGAUCCUUAUGAAGAUUUUCAAGAAAACUGG AACACUAAACAUAGCAGUGGUGUUAACCGUGAACUCAUGCGUGAGCUUAACG GAGGGUAAAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUC CCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAAAAAAAA AA AA
nvUTR- Barstar	GGGCGAAUUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGC CACCAUGGGAUCCAAAAAGCAGUCAUUAACGGGGAACAAUCAGAAGUAUC AGCGACCUCACCAGACAUUGAAAAAGGAGCUUGCCUGCCGGAUACUACG GUGAAAACCUGGACGCUUUAUGGGAUUGUCUGACCGGAUGGGUGGAGUACC CGCUCGUUUUGGAAUGGAGGCAGUUUGAACAAAGCAAGCAGCUGACUGAAA AUGGUGCCGAGAGUGUCUUCAGGUUUUCCGUGAAGCGAAAGCGGAAGGCU GCGACAUCACCAUCAUACUUCUAGGACCUUCUGCGGGGCUUGCCUUCUGG CCAUGCCCUUCUUCUCUCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGC CUGAGUAGGAAA AA AAAAAAAAAAAAAAAAAAAAA

sgUTR- Barnase	GAUUAAGGUUUUAUACCUUCCCAGGUAACAAACCAACCAACUUUCGAUCUCUUG UAGAUCUGUUCUCUAAACGAACAAACUAAAA AUGGGAUCCGCACAGGUUAUCAAC ACGUUUGACGGGGUUGCGGAUUAUCUUCAGACAUUAUCAUAAGCUACCUGAUAA UUACAUUACAAAUCAGAAGCACAAGCCCUCGGCUGGGUGGCAUCAAAGGGA ACCUUGCAGACGUCGCUCCGGGGAAAAGCAUCGGCGGAGACAUUCUUCUCAAAC AGGGAAGGCAAACUCCCGGGCAAAGCGGGCGAACAUGGCGUGAAGCGGAUA UUAACUUAUCAUCAGGCUUCAGAAAUUCAGACCGGAUUCUUUACUCAAGCGAC UGGCUGAUUUACAAAACAACGGACCAUUAUCAGACCUUUACAAAAUCAGUA GGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCAC CUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAA AA AA
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Note:

Sequences in **bold** are coding regions.

Supplementary Literature

- (1) Fujita, Y.; Hirose, M.; Hayashi, K.; Hatani, T.; Yoshida, Y.; Yamamoto, T.; Saito, H. A Versatile and Robust Cell Purification System with an RNA-Only Circuit Composed of microRNA-Responsive ON and OFF Switches. *Sci. Adv.* **2022**, *8* (1), eabj1793. <https://doi.org/10.1126/sciadv.abj1793>.