

Targeted Intracellular Degradation of SARS-CoV-2 via Computationally-Optimized Peptide Fusions

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1 Targeted Intracellular Degradation of SARS-CoV-2 2 via Computationally-Optimized Peptide Fusions

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4 **The COVID-19 pandemic, caused by the novel coronavirus SARS-**
5 **CoV-2, has elicited a global health crisis of catastrophic propor-**
6 **tions. With no approved cure or vaccine currently available, there**
7 **is a critical need for effective antiviral strategies. In this study, we**
8 **report a novel antiviral platform, through computational design of**
9 **ACE2-derived peptides which both target the viral spike protein**
10 **receptor binding domain (RBD) and recruit E3 ubiquitin ligases**
11 **for subsequent intracellular degradation of SARS-CoV-2 in the pro-**
12 **teasome. Our engineered peptide fusions demonstrate robust RBD**
13 **degradation capabilities in human cells and are capable of inhibiting**
14 **infection-competent viral production, thus prompting their further**
15 **experimental characterization and therapeutic development.**

16 Introduction

17 SARS-CoV-2 has emerged as a highly pathogenic coronavirus that has now spread to over
18 200 countries, infecting nearly 13 million people worldwide and killing over 560,000 as of
19 July 2020 (1). Economies have crashed, travel restrictions have been imposed, and pub-
20 lic gatherings have been cancelled, all while a sizeable portion of the human population
21 remains quarantined. Rapid transmission dynamics as well as a wide range of symptoms,
22 from a simple dry cough to pneumonia and death, are common characteristics of coron-
23 avirus disease 2019 (COVID-19) (2). With no vaccine or cure readily available (3), there
24 is a pressing need for robust and effective therapeutics targeting the virus.

25
26 Numerous antiviral strategies have been proposed to limit SARS-CoV-2 replication by
27 preventing viral infection and synthesis (4). As SARS-CoV-2 is a positive-sense RNA
28 virus, Abbott, et al. recently devised a CRISPR-Cas13d based strategy, termed PAC-
29 MAN, to simultaneously degrade the positive-sense genome and viral mRNAs (5). While
30 this method may serve as a potential prophylactic treatment, introducing foreign and rel-
31 atively large components such as Cas13 enzymes into human cells *in vivo* presents various
32 delivery and safety challenges (6).

33
34 The most rapid and acute method of protein degradation intracellularly is at the post-
35 translational level. Specifically, E3 ubiquitin ligases can tag endogenous proteins for
36 subsequent degradation in the proteasome (7). Thus, we hypothesize that by guiding E3
37 ubiquitin ligases to synthesized viral protein components, one can mediate depletion of
38 SARS-CoV-2 *in vivo*, preventing further infection and replication.

39

40 In this study, we devise a targeted intracellular degradation strategy for SARS-CoV-2
41 by computationally designing peptides that bind to its spike (S) protein receptor bind-
42 ing domain (RBD) and recruit a human E3 ubiquitin ligase for subsequent proteasomal
43 degradation. Our experimental results identify an optimal peptide variant that mediates
44 robust degradation of the RBD fused to a stable superfolder-green fluorescent protein
45 (sfGFP) (8) in human cells and inhibits infection-competent viral production, thus moti-
46 vating further exploration of this strategy from a therapeutic perspective.

47 **Results**

48 **Computationally-Optimized Peptides Targeting the SARS-CoV- 49 2 RBD**

50 Since the 2003 SARS epidemic, it has been widely known that the angiotensin-converting
51 enzyme 2 (ACE2) receptor is critical for SARS-CoV entry into host cells (9). ACE2 is a
52 monocarboxypeptidase, widely known for cleaving various peptides within the renin–angiotensin
53 system (10). Functionally, there are two forms of ACE2. The full-length ACE2 contains a
54 structural transmembrane domain, which anchors its extracellular domain to the plasma
55 membrane. The extracellular domain has been demonstrated as a receptor for the S
56 protein of SARS-CoV, and recently, for that of SARS-CoV-2 (11). The soluble form of
57 ACE2 lacks the membrane anchor, thus preserving binding capacity, and circulates in
58 small amounts in the blood (12).

59

60 Recently, it has been shown that soluble ACE2 (sACE2) can serve as a competitive inter-
61 ceptor of SARS-CoV-2 and other coronaviruses by preventing binding of the viral particle
62 to the endogenous ACE2 transmembrane protein, and thus viral entry (13). sACE2,
63 however, is capable of binding other biological molecules *in vivo*, most notably integrin

64 receptors (14). Furthermore, it is critical that therapeutics targeting SARS-CoV-2 epi-
65 topes withstand the possibility of viral mutation, which may allow the virus to overcome
66 the host adaptive immune response (15). We thus conducted *in silico* protein modeling
67 to engineer minimal sACE2 peptides that not only maintain potent RBD binding, but
68 also possess reduced off-target interaction with the integrin $\alpha 5\beta 1$ receptor, and exhibit
69 cross-binding affinity toward the previous SARS-CoV spike protein, thus demonstrating
70 tolerance to viral evolution (Figure 1A).

71

72 To do this, we retrieved a structure of the SARS-CoV-2 RBD bound to sACE2 from
73 the Protein Data Bank (PDB 6M0J) (16). We first utilized the PeptiDerive protocol (17)
74 in the Rosetta protein modeling software (18) to generate truncated linear sACE2 peptide
75 segments between 10 to 150 amino acids with significant binding energy compared to that
76 of the full SARS-CoV-2-sACE2-RBD interaction. To analyze the conformational entropy
77 of the peptide segments in the binding pocket, we employed both the FlexPepDock and
78 Protein-Protein protocols (19) to dock the peptides to the original RBD (Figure 1B.i).
79 To ensure tolerance to potential mutations in the RBD, we docked peptides with optimal
80 binding energies against the divergent 2003 SARS-CoV RBD bound with ACE2 (PDB
81 2AJF) (20) (Figure 1B.ii). Peptides that demonstrated highest binding energy for SARS-
82 CoV and SARS-CoV-2 RBD were then docked against the $\alpha 5\beta 1$ integrin ectodomain
83 (PDB 3VI4) (21) to identify weak off-target binders (Figure 1B.iii). After applying these
84 filters, 26 candidate peptides were selected from a total list of 188 initial peptides (Figure
85 1C).

86 Targeted Degradation of RBD with TRIM21

87 TRIM21 is an E3 ubiquitin ligase that binds with high affinity to the Fc domain of
88 antibodies and recruits the ubiquitin-proteasome system to degrade targeted proteins (22)

89 Recently, the Trim-Away technique was developed for acute and rapid degradation of
90 endogenous proteins, by co-expressing TRIM21 with an anti-target antibody (23). We

91 thus hypothesized that by fusing the Fc domain to the C-terminus of candidate peptides
92 and co-expressing TRIM21, we can mediate degradation of the RBD fused to a stable

93 fluorescent marker, such as superfolder GFP (8) (RBD-sfGFP), in human HEK293T cells
94 using a simple plasmid-based assay (Figure 2A and 2B). We chose the two most compact

95 candidate peptides, an 18-mer and 23-mer derived from the ACE2 peptidase domain $\alpha 1$
96 helix, which is composed entirely of proteinogenic amino acids, as well as the candidate

97 peptide computationally predicted to have highest binding affinity to the RBD (a 148-
98 mer), for testing alongside sACE2 (Figure 1C). We also tested a recently-engineered 23-

99 mer peptide from Zhang, et al., purporting to have strong RBD-binding capabilities (24).

100 Five days post-transfection, we analyzed the degradation of the RBD-sfGFP complex by
101 flow cytometry. After confirming negligible baseline depletion of GFP+ signal with and

102 without exogenous TRIM21 expression, as well as no off-target degradation of sfGFP
103 unbound to the RBD, we observed over 30% reduction of GFP+ cells treated with full-

104 length sACE2 fused to Fc and co-expressed with TRIM21, as compared to the RBD-
105 sfGFP-only control. Of the tested peptides, only the 23-mer demonstrated comparable

106 levels of degradation, with nearly 20% reduction in GFP+ cells (Figure 2C).

107 **Engineering of an Optimal Peptide-Based Degradation Architec-** 108 **ture**

109 Recently, deep mutational scans have been conducted on sACE2 to identify variants with
110 higher binding affinity to the RBD of SARS-CoV-2 (25). Similarly, we conducted a com-
111 plete single point mutational scan for all 23 positions in the peptide using the ddG-backrub
112 script in Rosetta to identify mutants with improved binding affinity (26). For each muta-
113 tion, 30,000 backrub trials were performed to sample conformational diversity. The top
114 eight mutations predicted by this protocol were chosen for the experimental assay (Figure
115 3A.i), along with the top eight mutations predicted using an Rosetta energy function opti-
116 mized for predicting the effect of mutations on protein-protein binding (26) (Figure 3A.ii),
117 as well as the top eight mutational sites within the 23-mer sequence from deep mutational
118 scans of sACE2 (25) (Figure 3A.iii). Our results in the subsequent TRIM21 assay identi-
119 fied A2N, derived from the original Rosetta energy function, as the optimal mutation in
120 the 23-mer peptide, which achieved over 50% depletion of GFP+ cells, improving on both
121 the sACE2 and 23-mer architecture as well as that of a previously optimized full-length
122 mutant, sACE2v2.4 (Figure 3B).

123
124 Finally, numerous previous works have attempted to redirect E3 ubiquitin ligases by
125 replacing their natural protein binding domains with those targeting specific proteins
126 (27–29). In 2014, Portnoff, et al., reprogrammed the substrate specificity of a modular
127 human E3 ubiquitin ligase called CHIP (carboxyl-terminus of Hsc70-interacting protein)
128 by replacing its natural substrate-binding domain with designer binding proteins to gen-
129 erate optimized “ubiquibodies” or uAbs (30). To engineer a single construct that can
130 mediate SARS-CoV-2 degradation without the need for *trans* expression of TRIM21, we
131 fused the RBD-binding proteins to the CHIP Δ TPR modified E3 ubiquitin ligase domain

132 (Figure 3C). After co-transfection in HEK293T cells with the RBD-sfGFP complex, we
133 observed that the 23-mer (A2N) mutant peptide maintained equivalent levels of degra-
134 dation between the TRIM21 and CHIP Δ TPR fusion architecture, and was more potent
135 than that of sACE2, sACE2v2.4, and the original 23mer (Figure 3D).

136 **Inhibition of Infection-Competent Viral Production**

137 Finally, we sought to assess the efficacy of our 23-mer (A2N)-CHIP Δ TPR fusion against
138 viruses pseudotyped with the SARS-CoV-2 S protein. We introduced a plasmid encoding
139 our construct during lentiviral production with a ZsGreen expression plasmid, lentivirus
140 packaging plasmid, and an envelope protein plasmid encoding the S protein (Figure 4A).
141 After viral supernatant recovery, we infected HEK293T cells expressing inducible hACE2
142 in the presence or absence of doxycycline, and quantified infection as the percentage of
143 ZsGreen+ cells by flow cytometry. Our data show that our 23-mer (A2N)-CHIP Δ TPR
144 fusion reduces the infection rate of the pseudovirus by nearly 50% (Figure 4B).

145 **Discussion**

146 In this study, we have computationally truncated and engineered the human ACE2 re-
147 ceptor sequence to potently bind to the SARS-CoV-2 RBD. We have further identified an
148 optimized peptide variant that enables robust degradation of RBD-sfGFP complexes in
149 human cells, both in *trans* and in *cis* with human E3 ubiquitin ligases. Finally, we have
150 shown that our optimal fusion construct inhibits the production of infection-competent
151 viruses pseudotyped with the S protein of SARS-CoV-2.

152

153 While further testing contexts are needed, there may be certain advantages to our plat-
154 form as compared to the PAC-MAN strategy presented recently (5). First, both of the

155 peptide and E3 ubiquitin ligase components have been engineered from endogenous human
156 proteins, unlike Cas13d, which is derived from *Ruminococcus flavefaciens* bacteria, thus
157 potentially reducing the risk of immunogenicity. In terms of *in vivo* delivery as RNA or re-
158 combinant protein, Cas13d has an open reading frame (ORF) of nearly 1000 amino acids,
159 not including the guide RNAs needed for interference. The entire peptide-CHIP Δ TPR
160 ORF consists of just over 200 amino acids, which can be readily synthesized as a peptide
161 or be efficiently packaged for delivery in a lipid nanoparticle or adeno-associated virus
162 (AAV). Similarly, the peptide-CHIP Δ TPR complex can be utilized as a viral interceptor
163 extracellularly, as it can competitively bind the RBD to prevent entry via ACE2. In the
164 case that SARS-CoV-2 still infects the cell while bound to peptide-CHIP Δ TPR, the virus
165 would be immediately tagged for degradation in the proteasome.

166

167 In total, we envision that the strategy of utilizing a computationally-designed peptide
168 binder linked to an E3 ubiquitin ligase can be explored not only for SARS-CoV-2, but
169 also for other viruses that have known binding partners. Thus with further characteriza-
170 tion, the system presented here provides a potential new therapeutic platform in the fight
171 against COVID-19 and future emergent viral threats.

172 **Materials and Methods**

173 **Computational Peptide Design Pipeline**

174 PDB Structure 6M0J containing the crystal structure of SARS-CoV-2 spike receptor-
175 binding domain (RBD) bound with ACE2 was retrieved (16). The PeptiDerive proto-
176 col (17) in the Rosetta protein modeling software (18) was used to determine the linear
177 peptide segments between 10 to 150 amino acids with significant binding energy compared
178 to that of the whole SARS-CoV-2-ACE2-RBD interaction. To analyze the conformational

179 entropy of the peptide segments in the binding pocket, a combination of FlexPepDock
180 and Protein-Protein docking protocols (19) in Rosetta was used to dock the peptides to
181 the original receptor binding domain. All peptides were placed in the binding pocket of
182 SARS-CoV-2 RBD for local docking. Using FlexPepDock, 300 models were created for
183 each peptide and the top 15 models were selected to calculate the score. The peptides with
184 the best binding energies were docked against SARS-CoV RBD bound with ACE2 using
185 PDB 2AJF (20) containing the crystal structure of SARS-CoV spike receptor-binding
186 domain (RBD) bound with ACE2. Peptides that demonstrated highest binding energy
187 for SARS-CoV and SARS-CoV-2 RBD were docked against the $\alpha 5\beta 1$ integrin ectodomain
188 using the crystal structure provided by PDB 3VI4 (21).

189

190 The 23-mer peptide that showed high experimental binding affinity was selected for com-
191 putational mutagenesis. A complete single point mutational scan was run for all 23
192 positions in the peptide using ddG-backrub script in Rosetta. For each mutation, 30,000
193 backrub trials were run to sample conformational diversity. The top 8 mutations predicted
194 by this protocol were chosen for the experimental assay, along with the top 8 mutations
195 predicted using an Rosetta energy function optimized for predicting the effect of muta-
196 tions on protein-protein binding (26), as well as the top 8 mutational sites predicted by
197 Procko on sACE2 (25).

198 **Generation of Plasmids**

199 pcDNA3-SARS-CoV-2-S-RBD-sfGFP (Addgene #141184) and pcDNA3-SARS-CoV-2-S-
200 RBD-Fc (Addgene #141183) were obtained as gifts from Erik Procko. hACE2 (Addgene
201 #1786) was obtained as a gift from Hyeryun Choe. Respective peptide DNA coding se-
202 quences (CDS) were amplified from hACE2 via PCR and inserted using HiFi DNA Assem-

203 bly Master Mix (NEB) for Gibson Assembly into the pcDNA3-SARS-CoV-2-S-RBD-Fc
204 backbone linearized by digestion with NheI and BamHI. pLVX puro TRIM21-GFP (Ad-
205 dgene #1786) was obtained as a gift from Gaudenz Danuser. The TRIM21 CDS was am-
206 plified with overhangs for Gibson Assembly-mediated insertion into the pcDNA3-SARS-
207 CoV-2-S-RBD-Fc backbone linearized by digestion with NheI and XhoI. pcDNA3-R4-
208 uAb (Addgene #101800) was obtained as a gift from Matthew DeLisa. Candidate sACE2
209 sequences were amplified from hACE2 with overhangs for Gibson Assembly-mediated in-
210 sersion into linearized pcDNA3-R4-uAb digested with HindIII and EcoRI. Single amino
211 acid substitutions were introduced utilizing the KLD Enzyme Mix (NEB) following PCR
212 amplification with mutagenic primers (Genewiz). Assembled constructs were transformed
213 into 50 μ L NEB Turbo Competent *E. coli* cells, and plated onto LB agar supplemented
214 with the appropriate antibiotic for subsequent sequence verification of colonies and plas-
215 mid purification.

216 **Cell Culture and FACS Analysis**

217 HEK293T cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) sup-
218 plemented with 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% fetal bovine
219 serum (FBS). RBD-sfGFP (333 ng), peptide (333 ng), and E3 ubiquitin ligase (333 ng)
220 plasmids were transfected into cells as duplicates (2×10^5 /well in a 24-well plate) with
221 Lipofectamine 3000 (Invitrogen) in Opti-MEM (Gibco). For transfection conditions with
222 fewer required plasmids, we co-transfected cells with the necessary amounts of empty
223 pcDNA3.1 plasmid to obtain 999 ng of total DNA. After 5 days post-transfection, cells
224 were harvested and analyzed on an Attune[®] NxT Flow Cytometer (Thermo Fisher) for
225 GFP fluorescence (488-nm laser excitation, 530/30 filter for detection). Cells expressing
226 GFP were gated, and percent GFP+ depletion to the RBD-sfGFP only control were cal-

227 culated. All samples were performed in independent transfection duplicates (n=2), and
228 percentage depletion values were averaged. Standard deviation was used to calculate error
229 bars.

230 **Cell Line Generation**

231 Doxycycline-inducible hACE2 HEK293T/17 cells were made by co-transfection in a 6-
232 well plate of 2 μ g Piggy-Bac expression plasmid containing a doxycycline-inducible hACE2
233 gene and puromycin selection marker and 0.5 μ g a Super PiggyBac Transposase expression
234 vector. After 6 days of 2 μ g/ml puromycin selection, the cells were harvested and genomic
235 DNA was extracted for diagnostic PCR to confirm integration of the expression cassette.
236 Additionally, mRNA was extracted 24 hours post-doxycycline induction for use in qPCR
237 to measure hACE2 expression levels.

238 **Pseudovirus Infection Assay**

239 Pseudotyped lentivirus was produced according to Thermo Fisher Lipofectamine 3000
240 lentivirus production protocol. Briefly, HEK293T/17 cells were seeded at high density
241 in 10 cm dishes in lentivirus packaging media (Gibco Opti-Mem Reduced serum medium
242 and 5% heat inactivated FBS) 24 hours prior to transfection. All lentiviruses utilized 7
243 μ g of CMV-ZsGreen lentivirus expression plasmid (Addgene #124301) as well as 7 μ g
244 of the second generation lentivirus packaging plasmid psPAX2 (Addgene #12260). For
245 envelope proteins, 4 μ g of a positive control VSV-G envelope plasmid was used to make a
246 VSV-G pseudotyped LV, 4 μ g of the Promega Transfection carrier DNA was used instead
247 of envelope protein to make the Bald pseudotyped lentivirus, and 4 μ g of the SARS-CoV-2
248 Spike protein (Addgene #145032) was used to make SARS-CoV-2 Spike lentivirus. To
249 make the SARS-CoV-2 Spike + 23mer (A2N) pseudotyped lentivirus, 4 μ g of the 23-mer

250 (A2N) plasmid and 4 μg of SARS-Cov-2 Spike were added. After mixing the plasmids
251 with Lipofectamine 3000, P3000 reagent in Opti-Mem, HEK293T cells were transfected
252 and the media was changed after 6 hours. Lentivirus-infused media was then collected
253 at 24 hours post-transfection and 52 hours post-transfection. The virus-containing me-
254 dia was then centrifuged at 2000 rpm and the supernatant was filtered through a 45 μm
255 filter to remove cellular debris. The 24 ml of filtered viral supernatant was then further
256 concentrated to 1 ml using the Lenti-X viral concentrator by adding 3x Lenti-X, mixing,
257 incubating for 45 minutes at 4 C, then centrifuging for 45 minutes at 1,500 g. The viral
258 pellet was then resuspended in 1 ml of PBS and aliquoted for use in transduction.

259

260 Doxycycline-inducible hACE2 HEK293T/17 cells were made by transfection of a Piggy-
261 Bac expression plasmid containing a doxycycline-inducible hACE2 gene and a puromycin
262 selection marker and a PiggyBac transposase vector. After 6 days of 3 $\mu\text{g}/\text{ml}$ puromycin
263 selection, the cells were harvested and gDNA was extracted for diagnostic PCR to con-
264 firm integration of the expression cassette. Additionally, mRNA was extracted 24 hours
265 post-doxycycline induction for use in qPCR to measure hACE2 expression levels.

266

267 All viral transduction was performed in 24-well culture dishes. Briefly, control HEK293T/17s,
268 non doxycycline-induced hACE2 293T/17s and Dox-induced hACE2 HEK293T/17s were
269 plated at high density and were 50% confluent at the time of transduction. Viral aliquots
270 were thawed for 2 minutes at 37 degrees prior to use and were not subjected to multiple
271 freeze-thaw cycles. In duplicate, 80 μL of concentrated virus was added to each well of the
272 293Ts in media containing 5 $\mu\text{g}/\text{ml}$ polybrene. Additionally, for the doxycycline-induced
273 cells, 1 $\mu\text{g}/\text{ml}$ of doxycycline was added along with the virus. After 36 hours the cells
274 were harvested for flow cytometry and percentage of ZsGreen+ cells was calculated in

275 each condition.

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279 Labs for access to flow cytometry.

280 **Author Contributions**

281 P.C. conceived, designed, and supervised the study. P.C. designed and built constructs,
282 carried out experiments, and conducted data analyses. M.P. implemented computational
283 design pipelines and performed *in silico* docking protocols. C.K. and A.M.P generated
284 cell lines and conducted pseudovirus assays. P.C. wrote the paper, with input from M.P.,
285 C.K., and A.M.P. G.M.C. supervised pseudovirus work. J.M.J. reviewed the paper and
286 provided critical insight and ideas.

287 **Declarations**

288 This work was supported by the consortia of sponsors of the MIT Media Lab and the
289 MIT Center for Bits and Atoms, and by Jeremy and Joyce Wertheimer. P.C. and J.M.J.
290 are listed inventors for provisional patent application entitled "Minimal Peptide Fusions
291 for Targeted Intracellular Protein Degradation."

292 **Data and Materials Availability**

293 All data needed to evaluate the conclusions in the paper are present in the paper. Raw
294 computational and experimental data files can be found at: shorturl.at/bipH3.

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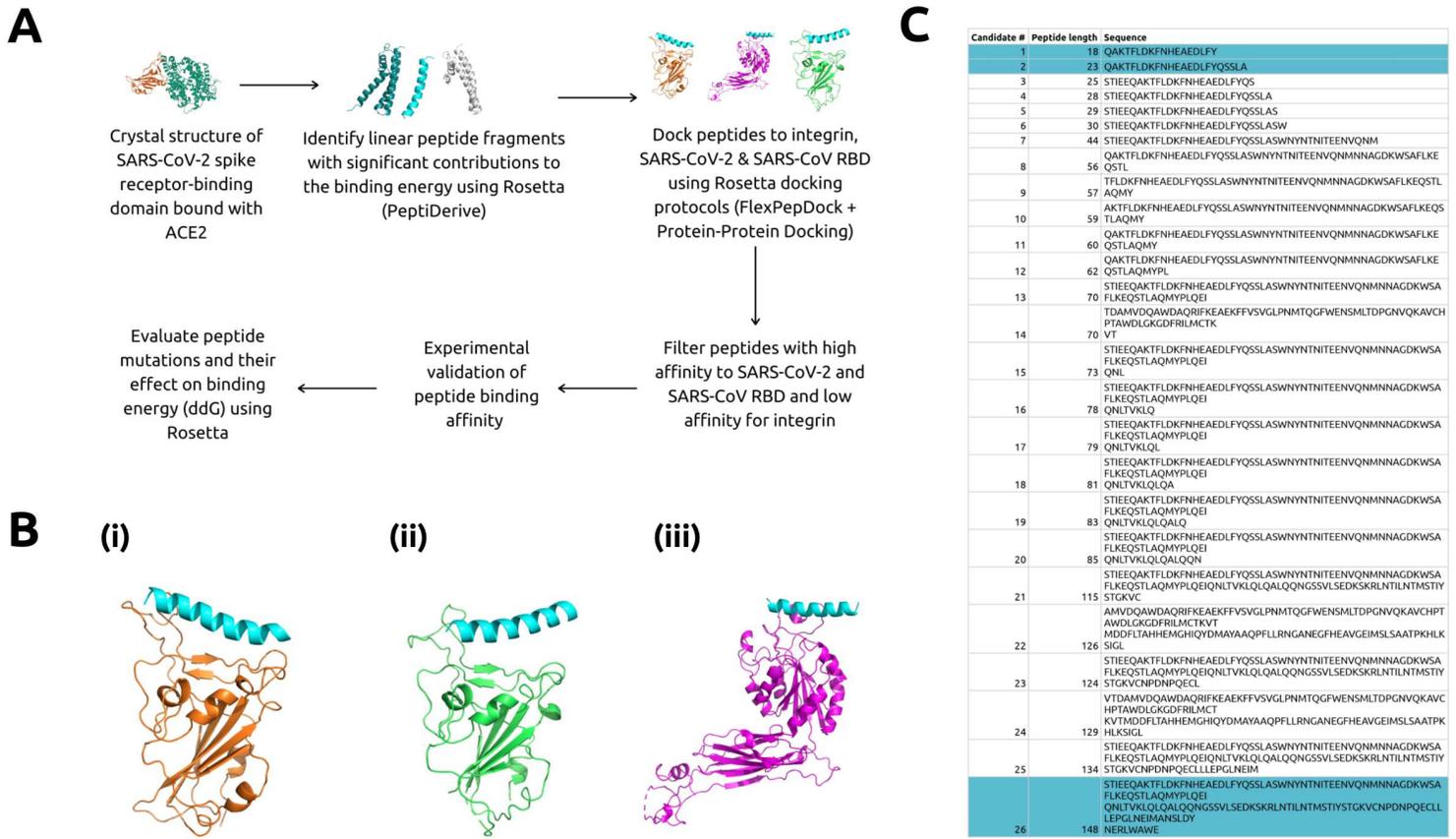


Figure 1: *In silico* Design of RBD-Targeting Peptides. A) Flow chart detailing computational pipeline to obtain optimized peptides. B) 23-mer peptide computationally docked to (i) SARS-CoV-2 RBD (16), (ii) SARS-CoV RBD (20), and (iii) integrin $\alpha 5 \beta 1$ receptor (21) in Rosetta and visualized using PyMol. The 23-mer peptide is shaded in blue. C) Candidate peptides selected after application of three filter docking steps. Peptides highlighted in blue indicate those chosen for experimental validation.

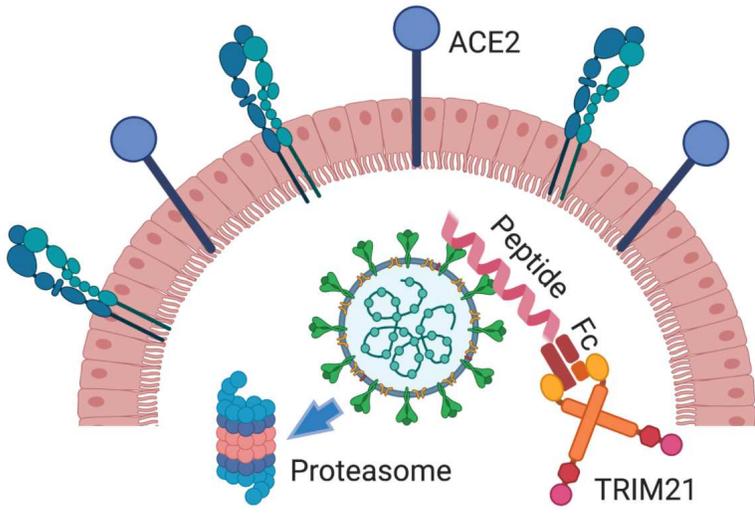
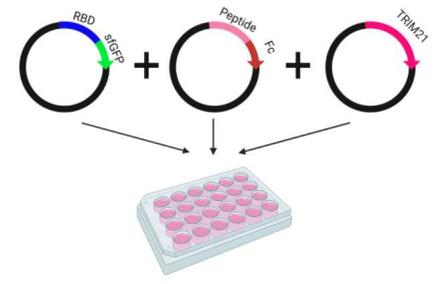
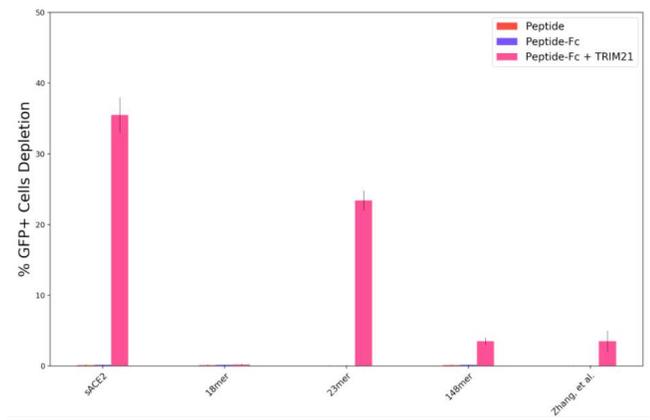
A**B****C**

Figure 2: TRIM21-mediated Degradation of RBD via Peptide Targeting. A) Architecture and mechanism of TRIM21-based degradation system. The Fc domain is fused to the C-terminus of RBD-targeting peptides. TRIM21 recognizes the Fc domain and tags SARS-CoV-2 for ubiquitin-mediated degradation in the proteasome. B) Three plasmid assay used to experimentally validate degradation architecture in human HEK293T cells. All CDS are inserted into the pcDNA3.1 backbone. C) Analysis of RBD-sfGFP degradation by flow cytometry, in the absence or presence of Fc (*in cis*), TRIM21 (*in trans*), or both. All samples were performed in independent transfection duplicates (n=2) and gated on GFP+ fluorescence. Mean percentage of GFP+ cell depletion was calculated in comparison to the RBD-sfGFP only control. Standard deviation was used to calculate error bars.

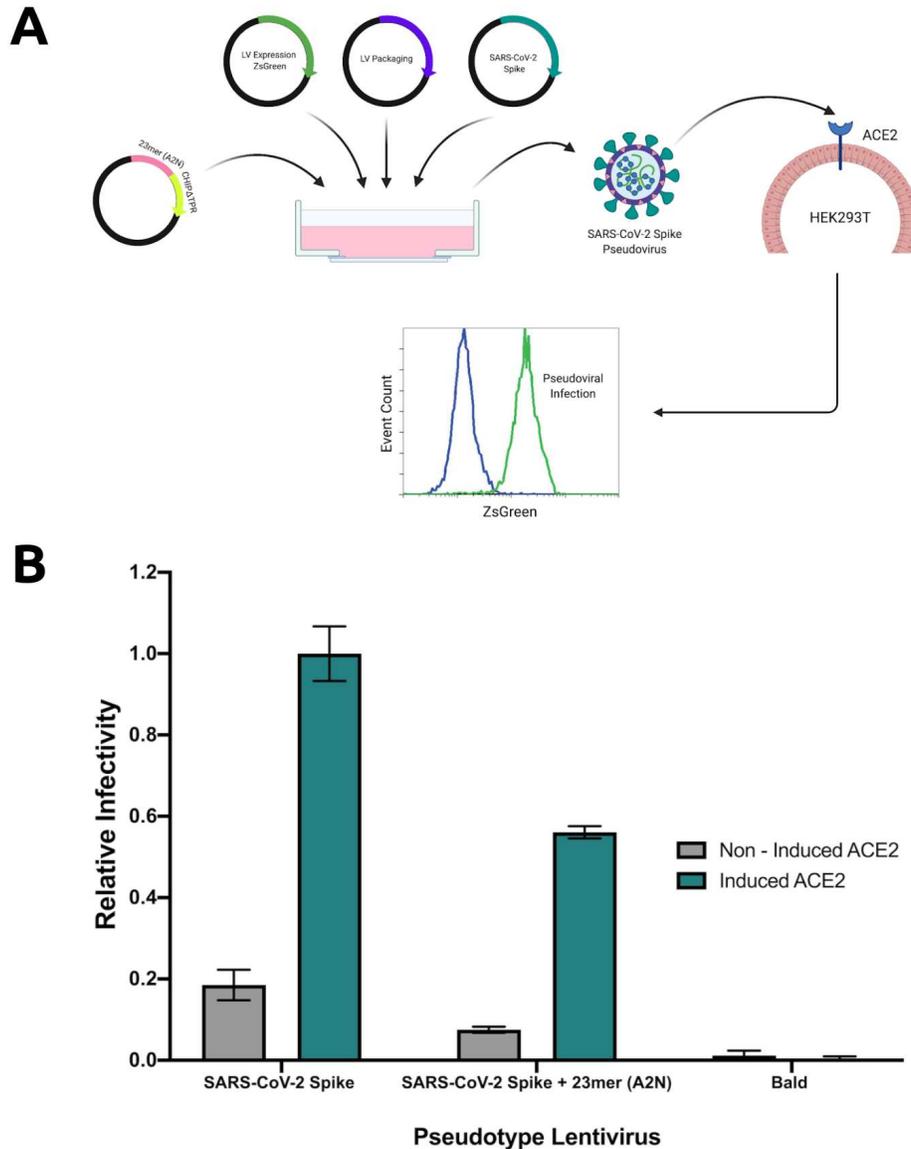


Figure 4: Inhibition of Infection-Competent Viral Production. A) Schematic demonstrating repurposed pseudoviral infection assay. Pseudotyped lentivirus is produced with a ZsGreen expression plasmid, lentivirus packaging plasmid, and an envelope protein plasmid encoding the S protein, in the presence of the (A2N)-CHIP Δ TPR fusion plasmid. After viral supernatant recovery, hACE2 expressing HEK293T cells are infected, and infection rate is calculated as the percentage of ZsGreen+ cells by flow cytometry. B) Analysis of pseudoviral infection degradation by flow cytometry. All samples were performed in independent transfection duplicates (n=2) and gated on ZsGreen+ fluorescence. Relative infectivity ratios were calculated by normalizing ZsGreen+ fluorescence percentages to the SARS-CoV-2 Spike (induced ACE2) positive control. Standard deviation was used to calculate error bars.

Figures

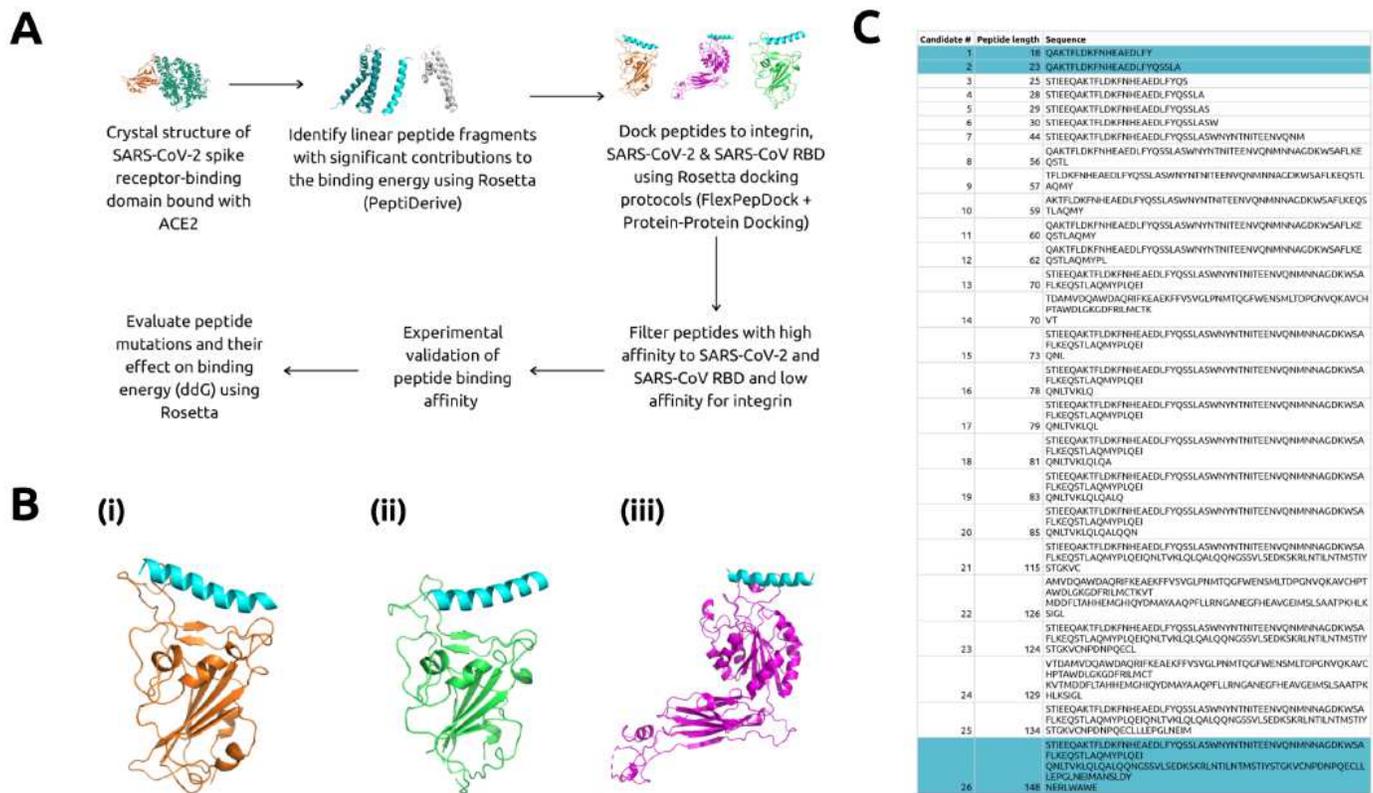


Figure 1

In silico Design of RBD-Targeting Peptides. A) Flow chart detailing computational pipeline to obtain optimized peptides. B) 23-mer peptide computationally docked to (i) SARS-CoV-2 RBD (16), (ii) SARS-CoV RBD (20), and (iii) integrin $\alpha 1\beta$ receptor (21) in Rosetta and visualized using PyMol. The 23-mer peptide is shaded in blue. C) Candidate peptides selected after application of three filter docking steps. Peptides highlighted in blue indicate those chosen for experimental validation.

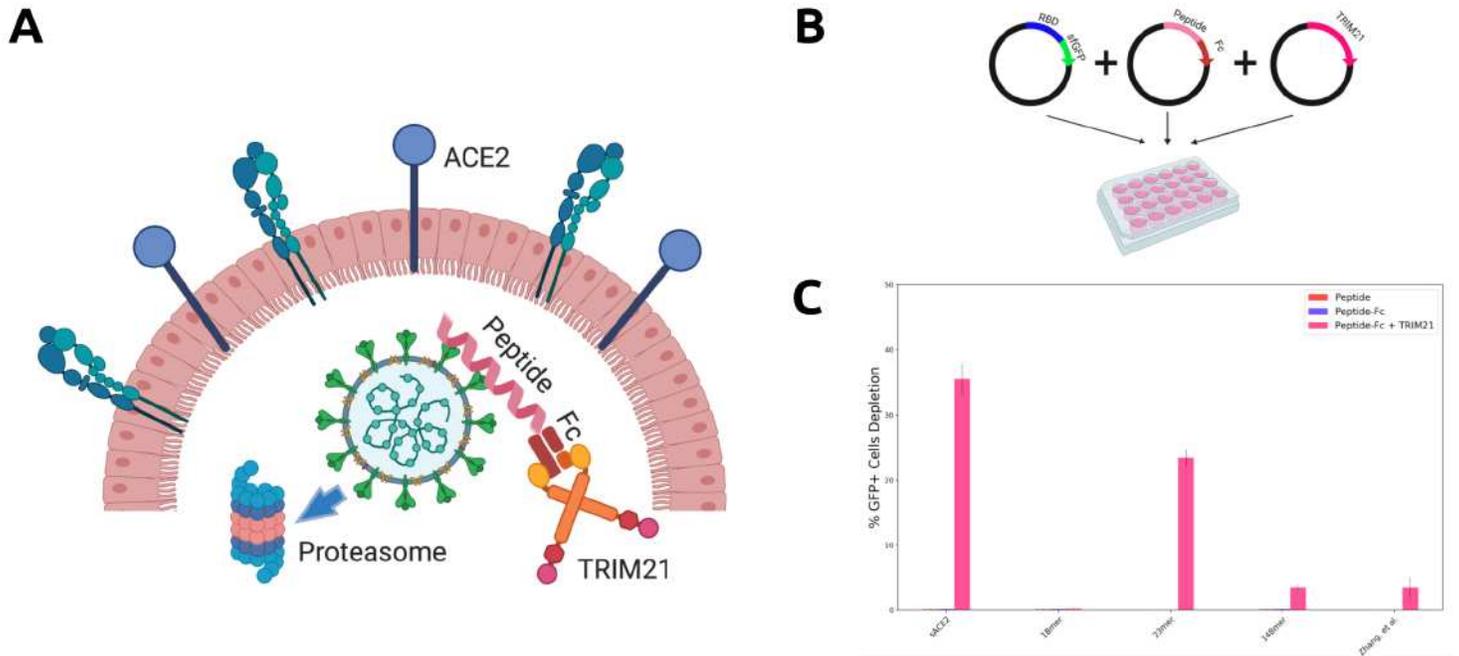


Figure 2

TRIM21-mediated Degradation of RBD via Peptide Targeting. A) Architecture and mechanism of TRIM21-based degradation system. The Fc domain is fused to the C-terminus of RBD- targeting peptides. TRIM21 recognizes the Fc domain and tags SARS-CoV-2 for ubiquitin-mediated degradation in the proteasome. B) Three plasmid assay used to experimentally validate degradation architecture in human HEK293T cells. All CDS are inserted into the pcDNA3.1 backbone. C) Analysis of RBD-sfGFP degradation by ow cytometry, in the absence or presence of Fc (in cis), TRIM21 (in trans), or both. All samples were performed in independent transfection duplicates (n=2) and gated on GFP+ fluorescence. Mean percentage of GFP+ cell depletion was calculated in comparison to the RBD-sfGFP only control. Standard deviation was used to calculate error bars. 19

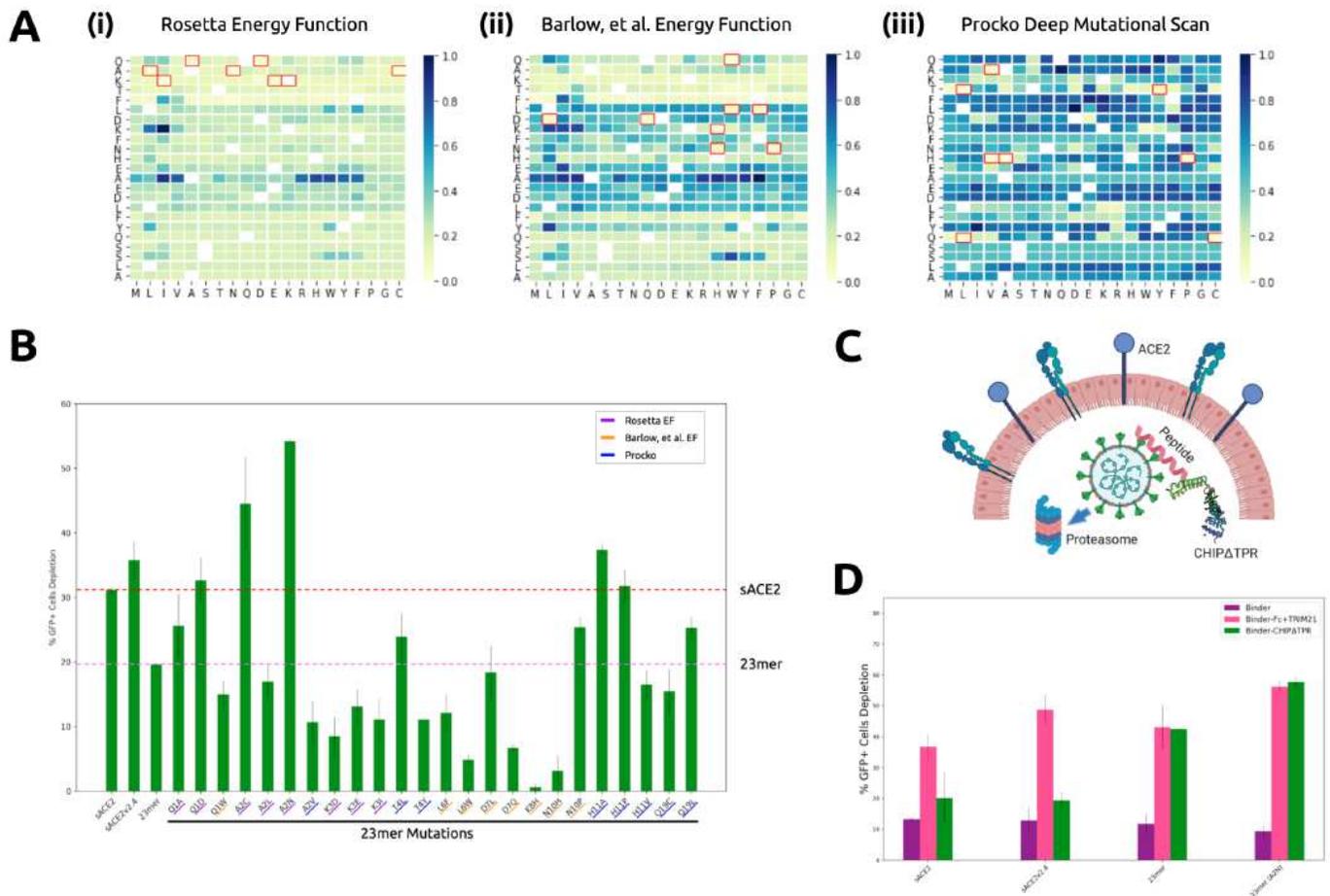


Figure 3

Engineering of Optimal RBD Degradation Architecture. A) (i) ddG of 23-mer mutations predicted by Rosetta with the default energy function, (ii) ddG of 23-mer mutations predicted by Rosetta with the energy function from Barlow et al. (26), (iii) log₂fc enrichment scores of mutations of sACE2 within the 23-mer sequence experimentally determined by Procko (25). All binding affinity scores have been re-scaled to 0 (highest) to 1 (lowest) for visualization. Original amino acids are indicated on the y-axes. B) Analysis of RBD-sfGFP degradation by ow cytometry. All samples were performed in independent transfection duplicates (n=2) and gated on GFP+ fluorescence. All indicated samples were co-transfected with RBD-sfGFP and TRIM21 in trans, and mean percentage of GFP+ cell depletion was calculated in comparison to the RBD-sfGFP-only control. Standard deviation was used to calculate error bars. 23-mer mutations are underlined according to origin. C) Architecture and mechanism of CHIP Δ TPR-based degradation system. CHIP Δ TPR is fused to the C-terminus of RBD-targeting peptides. CHIP Δ TPR can thus tag SARS-CoV-2 for ubiquitin-mediated degradation in the proteasome. D) Analysis of RBD-sfGFP degradation by ow cytometry in the presence of Fc (in cis) and TRIM21 (in trans) or CHIP Δ TPR (in cis). All samples were performed in independent transfection duplicates (n=2) and gated on GFP+ fluorescence. Mean percentage of GFP+ cell depletion was calculated in comparison to the RBD-sfGFP only control. Standard deviation was used to calculate error bars.

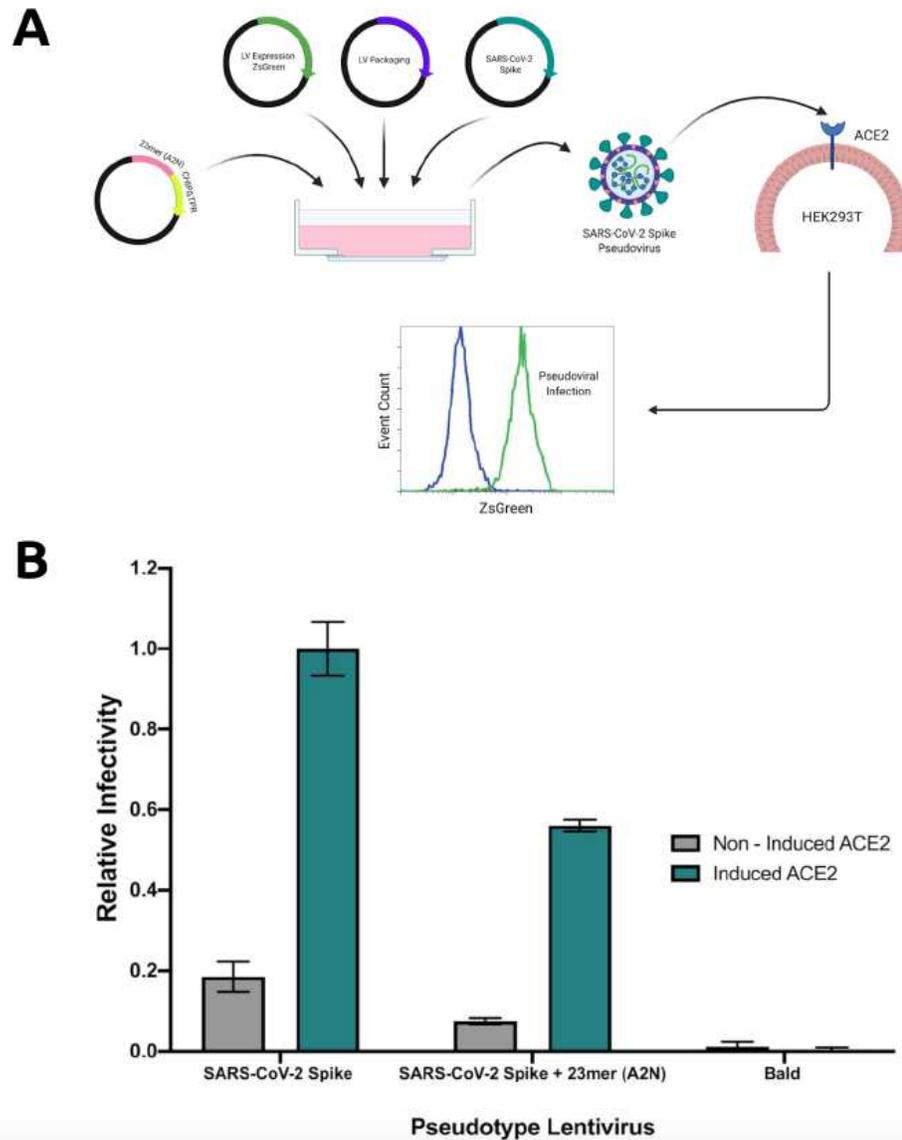


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