

## Supplementary Materials for

### **Development of a fluorescence based, high-throughput SARS-CoV-2 3CL<sup>pro</sup> reporter assay**

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Fig. S1. Modified FlipGFP construct including NheI site

Fig. S2. Images of background FlipGFP fluorescence

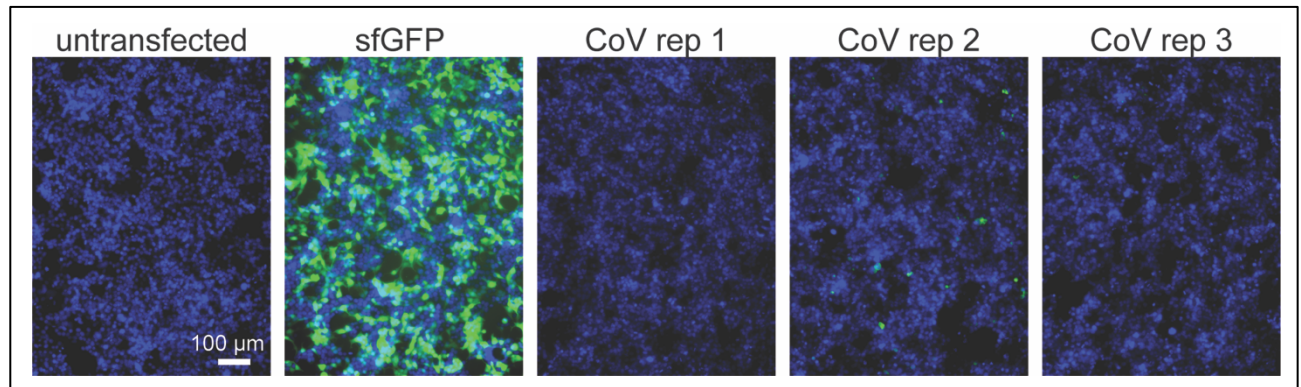
Table S1. Primers for CoV 3CL<sup>pro</sup> responsive FlipGFP constructs

**Fig. S1.**

FlipGFP (modified)	1065 bp ds-DNA	linear				
BamHI	1..6					
FlipGFP	13..1077					
NheI	268..2273					
modified nucleotides	270..273, 1062, 1065					
TEV cleavage site	274..294					
	/product="tobacco etch virus (TEV) protease recognition and cleavage site"					
	/translation="ENLYFQS"					
2A	406..459					
NotI	1078..1085					
<b>SEQUENCE</b>						
1	ggatccgcc	ccatggacct	gcctgacgac	cactacctgt	ccaccagac	catcctgtcc
61	aaggacctga	actccggact	cagatctggc	agcggctctcg	agatggaagt	tagcgcctctg
121	gaaaaagaag	tgtctgcaact	cgagaaagaa	gtaagtgcc	ttgagaagga	ggtgtccgca
181	ctcgagaagg	aggtcagcgc	cctggaaaag	gaaaagcgag	accataggt	tttgcttgag
241	tatgttacag	cggctggcat	taccgatgcT	AGCgaaaacc	tgtactttca	gtccaagggtg
301	tccgccctga	aggaaaaagt	aagcgcactg	aaagaaaagg	tgagcgcgct	gaaggagaaa
361	gtgagcgcgc	tgaaagagaa	agtctctgcc	cttaaggagg	atatacgagg	gagaggaagt
421	ctgctaacat	gcggtgacgt	cgaggagaa	cctggcccaa	agcttgccac	catgcgcaaa
481	ggcgaagaac	tgtttaccgg	cattgtgccc	attctggtgg	aactggatgg	cgatgtgaac
541	ggcataaat	tttttgtgcg	cggcgaaggc	gaaggcgatg	cgaccattgg	caaactgagc
601	ctgaaattta	tttgcaccac	cggcaaactg	ccgggtgccg	ggccgaccct	ggtgaccacc
661	ctgacctatg	gcgtgcagtg	ctttagccgc	tatccggatc	atatgaaacg	ccatgatctt
721	tttaaaagcg	cgatgccgga	aggctatgtg	caggaacgca	ccatttattt	taaagatgat
781	ggcacctata	aaaccgcgc	ggaagtgaaa	tttgaaggcg	ataccctgg	gaaccgcatt
841	gaactgaaag	gcattgattt	taaagaagat	ggcaacattc	tgggccataa	actggaatat
901	aactttaaca	gccataaagt	gtatattacc	gcggataaac	agaacaacgg	cattaaagcg
961	aactttacca	ttcgccataa	cgtggaagat	ggcagcgtgc	agctggcgga	tcattatcag
1021	cagaacacc	cgattggcga	tggcccgggt	cttcttctcg	gAggAcgctc	tagataggcg
1081	gccgc					

**Fig. S1. Modified FlipGFP construct.** Nucleotide sequence of the modified FlipGFP construct used in this study. Capital letters indicate nucleotide changes introduced to silently create a new *NheI* site and eliminate an existing *NotI* site within FlipGFP.

**Fig. S2.**



**Fig. S2. Images of background FlipGFP fluorescence.** Images of 293T cells 48 hours post-transfection with each FlipGFP reporter or sfGFP. Green = fluorescing GFP, blue = nuclei. Scale bars are 100μm. Experiment performed twice.

**Table S1.** Primers for CoV 3CL<sup>pro</sup> responsive FlipGFP constructs

<b>Name</b>	<b>Sequence</b>
FlipGFP For	gaagacaccgactctactagaggatccgccaccatggacctgcctgacgaccac
FlipGFP Rev	cagacgcgtcggggccctctagactcgagcggccgcctatctagagcgTccTcc
CoV rep 1 For	gctggcattaccgatgcTAGCagcgcctgctgcagagcggcttcaaggtgtccgccctgaa
CoV rep 2 For	gctggcattaccgatgcTAGCaccgtgaagctgcaggccggcttcaaggtgtccgccctgaa
CoV rep 3 For	gctggcattaccgatgcTAGCgtggccagactgcagagcggcttcaaggtgtccgccctgaa