

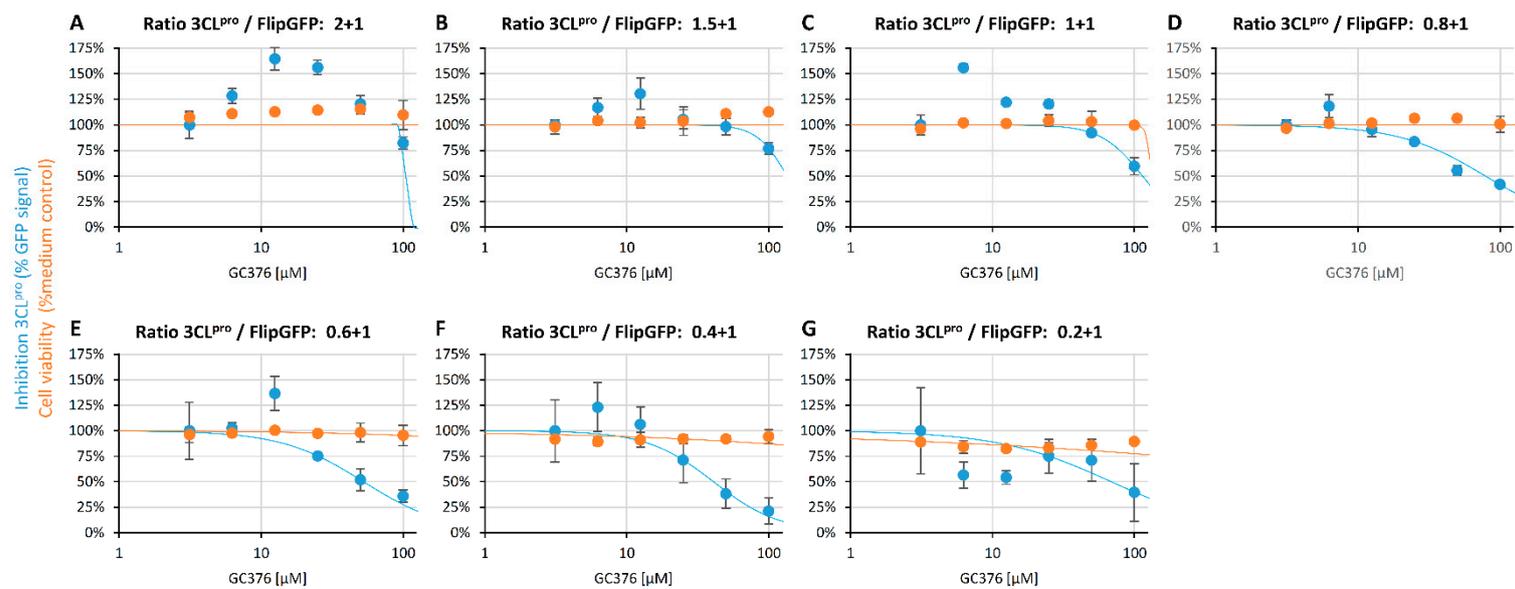
# Methodological development of a multi-readout assay for the assessment of antiviral drugs against SARS-CoV-2

Friedrich Hahn <sup>1</sup>, Sigrun Häge <sup>1</sup>, Alexandra Herrmann <sup>1</sup>, Christina Wangen <sup>1</sup>, Jintawee Kicuntod <sup>1</sup>, Doris Jungnickl <sup>1</sup>, Julia Tillmanns <sup>1</sup>, Regina Müller <sup>1</sup>, Kirsten Fraedrich <sup>1</sup>, Klaus Überla <sup>1</sup>, Hella Kohlhof <sup>2</sup>, Armin Ensser <sup>1</sup> and Manfred Marschall <sup>1,\*</sup>

- 1 Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg (FAU), Erlangen, Germany; friedrich.hahn@uk-erlangen.de (F.H.); sigrun.haegel@fau.de (S.H.); alexandra.herrmann@uk-erlangen.de (A.H.); christina.wangen@uk-erlangen.de (C.W.); jintawee.kicuntod@extern.uk-erlangen.de (J.K.); doris.jungnickl@uk-erlangen.de (D.J.); jul.tillmanns@fau.de (J.T.); mueller.regina@uk-erlangen.de (R.M.); kirsten.fraedrich@uk-erlangen.de (K.F.); klaus.ueberla@fau.de (K.Ü.); armin.ensser@fau.de (A.E.); manfred.marschall@fau.de (M.M.)
  - 2 Immunic AG, Gräfelfing, Germany; hella.kohlhof@imux.com (H.K.)
- \* Correspondence: manfred.marschall@fau.de; phone +49 9131 8526089

**Table S1.** Oligonucleotide primers used in this study. The following information is given by the sequence description: translational start or stop codons (capital letters, underlined), restriction sites (capital letters, bold), additional bases (lower case letters) and coding sequences (capital letters).

Primer	Sequence (5' → 3')
5-BamHI-eCFP	tag <b>GGATCC</b> ATGGTGAGCAAGGGCGAGGAG
3-XbaI-NotI-eYFP	tag <b>TCTAGA</b> ctcga <b>GCGGCCGCTT</b> ACTTGTACAGCTCGTCCATG
5-3CL pro Cleavage-SpeI-eYFP	<b>GCGCTAG</b> CGTGGCCAGACTGCAGAGCGGCTT <b>CACTAGT</b> GGCAGCGTGAGCAAGGGCGAGGAG
3-3CL pro Cleavage-NheI-eCFP	CCACTAGTGAAGCCGCTCTGCAGTCTGGCCAC <b>GCTAG</b> CGCTGCCCTTGTACAGCTCGTCCATGC
5-T2A Cleavage-eYFP	GGGCAGCCTGCTGACCTGCGGCGACGTGGAGGAGAAACCCCGGCCCGTGAGCAAGGGCGAGGAG
3-T2A Cleavage-eCFP	GGGGTTCTCCTCCACGTCGCCGAGGTCAGCAGGCTGCCCGGCCCTCGCCGCTGCCCTGTACAGCTCGTCCATG



**Figure S1:** Steps of optimization of the FlipGFP assay: protease/reporter ratios (A-G). Several different ratios of transfected plasmids coding for the protease or reporter protein, respectively, were applied as indicated.