Supplementary information

A natural product compound inhibits coronaviral replication *in vitro* by binding to the conserved Nsp9 SARS-CoV-2 protein

Dene R. Littler [1]*, Miaomiao Liu[2], Julie L. McAuley[3], Shea A. Lowery[4], Patricia T. Illing[1], Benjamin S. Gully[1], Anthony W. Purcell[1], Indu R. Chandrashekaran[5], Stanley Perlman[4, 6], Damian F. J. Purcell[3], Ronald J. Quinn[2,3*] & Jamie Rossjohn[1,7*]

[1] Infection and Immunity Program & Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia. [2] Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD, Australia.[3] Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, VIC, Australia.[4] Department of Microbiology and Immunology, University of Iowa, Iowa City, Iowa, USA.[5] Monash Institute of Pharmaceutical Sciences, Monash University, Royal Parade, Parkville, Victoria, Australia. [6] Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA. [7] Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff, United Kingdom.

	Oridonin:3C-Nsp9 $_{\text{COV19}}$
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	84.01, 88.25, 85.95
α, β, γ (°)	90.0, 90.0, 90.0
Resolution (Å)	88.25-3.00 (3.16-3.00)
R_{pim}[1]	3.5 (52.5)
R_{merge}	6.3 (92.6)
I/σ_1	10.5 (1.5)
$CC_{1/2}$	0.999 (0.541)
Completeness (%)	99.1 (99.6)
Total N$^{o.}$ observations	76786 (10764)
N$^{o.}$ unique observations	25089 (3682)
Multiplicity	3.1 (2.9)
Refinement statistics	
R_{factor}[2] (%)	22.8
R_{free}[3] (%)	28.7
No. atoms	
• Protein	7560
• Water	n/a
• Ligands	8 ODN, 4 SO_4
Ramachandran plot (%)	
• Most favoured	92.1
• Allowed region	8.0
• Outlier	0.1 (Pro C -1)
B-factors (Å^2) – Protein (oridonin)	102.8 (95.8)
rmsd bonds (Å)	0.011
rmsd angles (°)	1.51

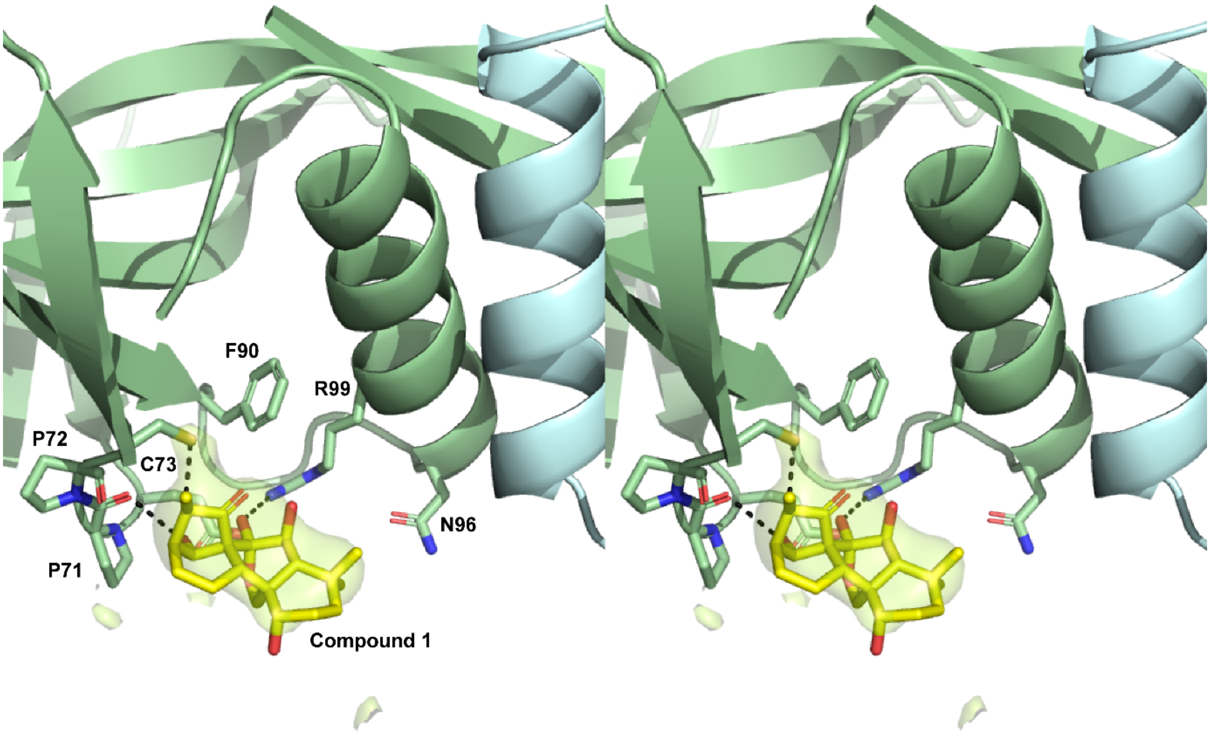
Supp table 1 Data collection and refinement statistics

[1] $R_{p.i.m} = \Sigma_{hkl} [1/(N-1)]^{1/2} \Sigma_i \mid I_{hkl,i} - \langle I_{hkl} \rangle \mid / \Sigma_{hkl} \langle I_{hkl} \rangle$

[2] $R_{factor} = (\Sigma \mid |F_o| - |F_c| \mid) / (\Sigma |F_o|)$ - for all data except as indicated in footnote 3.

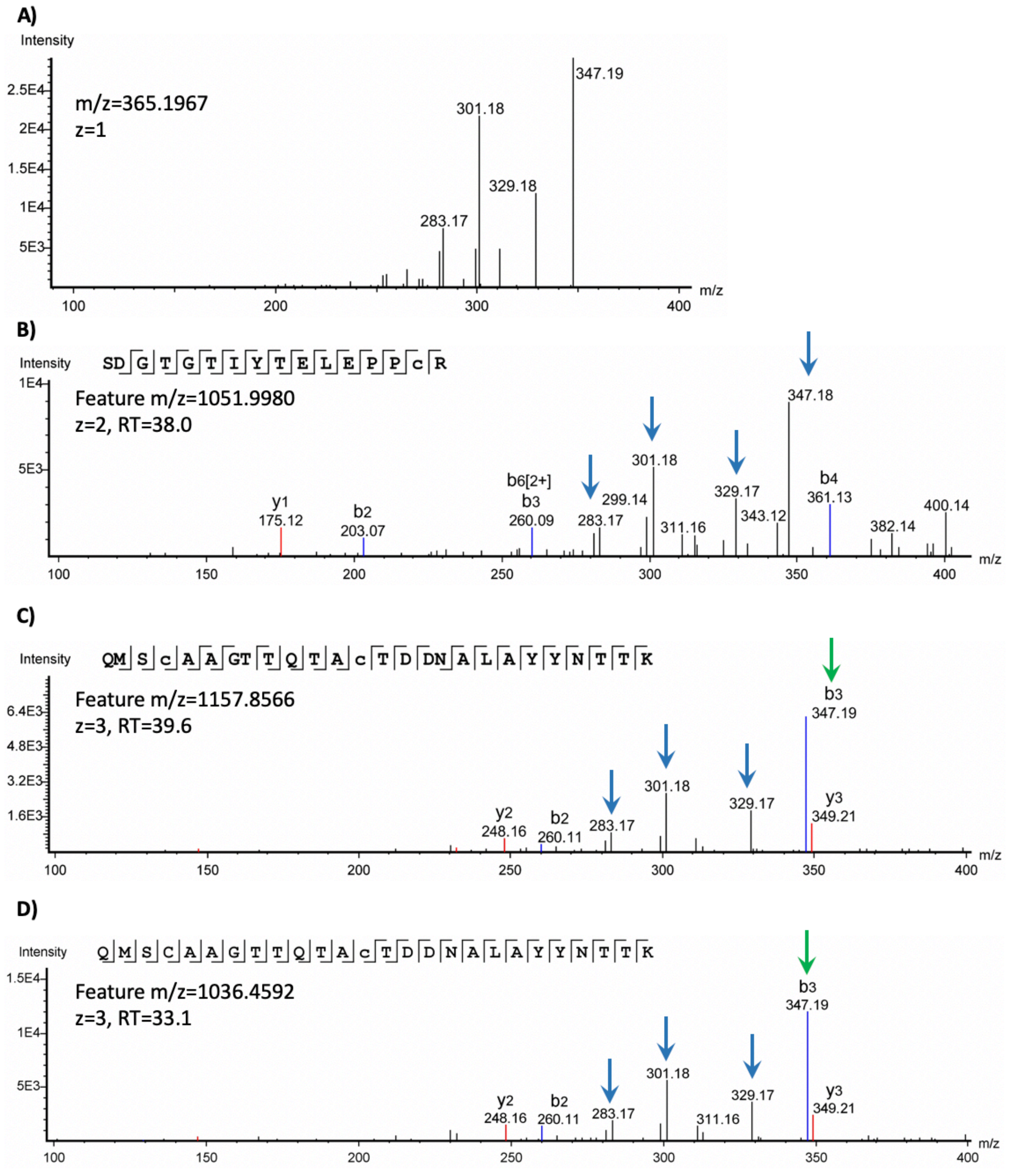
[3] 5% of data was used for the R_{free} calculation

Values in parentheses refer to the highest resolution bin



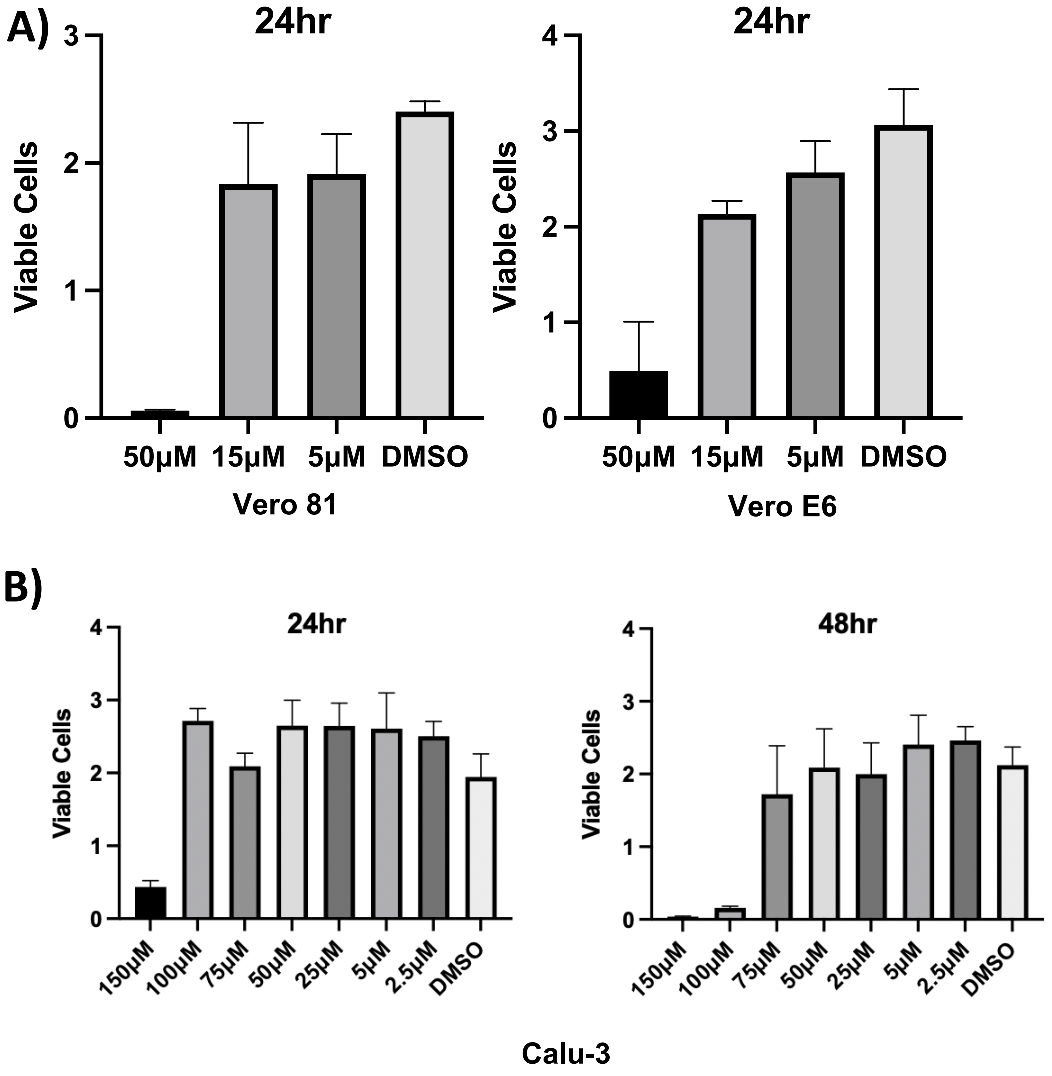
Supplementary Figure 1

Stereo Omit map contoured at 3σ within the Compound 1 binding site of Nsp9$_{COV19}$ prior to refinement of the molecule within the site. Colours used are consistent with those of Figure 2A.



Supplementary figure 2:

A) shows fragmentation of oridonin, including prominent ions at m/z 347.19, 329.18, 301.18 and 283.17. **B-D**) Lower mass regions of fragmentation spectra of modified peptides, including fragment ions consistent with fragmentation of oridonin(blue arrows). Note, for QMSCAAGTTQTACTDDNALAYYNTTK, 347.19 is also the mass of peptide fragment b3 (QMS) (green arrows). Spectra were extracted from PEAKS X Pro, lower case c denotes the Cys residue(s) modified with oridonin. RT denotes retention time.



Supplementary Figure 3

MMT proliferation assay of different cell types treated with different concentrations of compound **1**. Error-bars represent S.D. of three independent replicates. Cells include **A)** Vero cells and **B)** a wider concentration of compound **1** in Calu-3 cells at the 24 and 48hr HPI (in which a broader range of concentrations was tested).