
Supplementary information

Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing

In the format provided by the
authors and unedited

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Supplementary Discussion

Large-scale surveys of existing drugs that may harbor antiviral activities can significantly facilitate repositioning efforts to identify efficacious treatments for COVID-19. This work reports the high-throughput analysis of approximately 12,000 known drugs evaluated for activity against SARS-CoV-2 replication. The assay, conducted in Vero E6 cells was designed to capture multicycle replication, based upon low viral input (MOI = 0.01) and an extended endpoint measurement (72 hours post-infection). To select candidates for validation studies, compounds were ranked according to their Z-score in the primary screen replicates (**Figure 1b-d**). While the average Z' factor of the first replicate was determined to be 0.51, the duplicate ReFRAME screen harbored a 40% reduction in dynamic range and corresponding Z' factor (0.19). Importantly, the correlation between the two screens was high ($R^2 = 0.68$), but, as expected, there were compounds that were found active in replicate 1, but not replicate 2. For this reason, data from replicate 1 was weighted more heavily, with 100 compounds selected exclusively from the replicate 1 dataset. Additionally, 75 compounds were selected based on average scores between the two replicates, 75 compounds were selected that were only found to be highly active in set 2, while the 48 remaining compounds were selected based on inclusion in one of the enriched GSEA categories (**Figures 2a and ED2**). These selected compounds were tested in an orthogonal assay that directly measures viral replication in contrast to the indirect measurement of replication assessed by CPE.

As noted in the main text, the immunostaining endpoint utilized in the validation screen enabled the separation of molecules that function to block CPE (i.e. cell death) from those with direct effects on replication. In addition, these validation assays were conducted employing lower drug concentrations than were utilized in the original screen (5 μ M). Thus, these more stringent conditions likely removed molecules that either function to block viral-induced cell death or only function at high concentrations, both of which are unlikely to be useful in a therapeutic setting. The introduction of the described stringencies during the validation step, as well as false positive activities from the HTS assay, likely account for confirmation rates observed at this step of the analysis.

Importantly, the secondary (validation) assay was found to be most robust at a 24-hour timepoint using an MOI of 0.1, in contrast to the 72 hour endpoint with a viral MOI input of 0.01 employed in the screen. This likely biased the validation screen towards the confirmation of early stage inhibitors. Consistent with this hypothesis, we find that several molecules with potent EC₅₀s were only able to inhibit replication levels to approximately 50-60% even at high concentrations, including MLN-3897, YH-1238 and SL-11128 (**Figures 3a-b and ED6c**). While this may represent the maximal ability of these molecules to suppress viral replication, an alternative hypothesis is that these molecules work at later stages of replication. Specifically, late acting molecules will not be able to prevent the first round of detectable infection (i.e. NP synthesis after a first wave of incoming virus), but only subsequent viral spread, and thus the maximal inhibition of infection would not be expected to reach 80-100%. Analysis of potential late-stage molecules utilizing lower MOIs at later timepoint may reveal greater inhibition of infection.

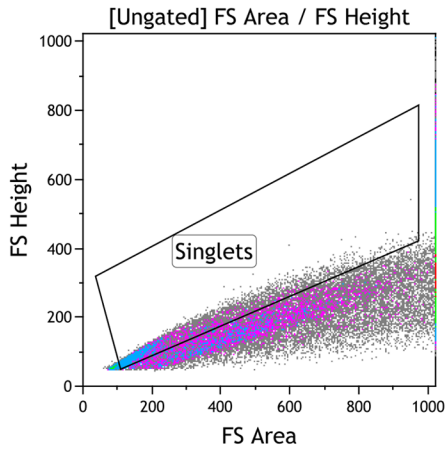
One potential limitation of employing Vero E6 cells derived from African green monkeys in the HTS assay is that species-specific differences may impact the results. For example, drugs that require the human host cell machinery for processing into their active form, such as some nucleoside inhibitors, may not harbor the same potency as in human cells. Consistently, we found that remdesivir inhibits SARS-CoV-2 replication ~60-fold more potently in human cells in comparison to Vero E6 cells (**Figures ED6c and ED7**). In contrast to direct acting antivirals, the efficacies of host-targeted therapies are reliant upon the disruption of specific cellular networks that govern host-pathogen interactions during infection, and thus can be cell-type dependent. Therefore, we further investigated if the observed antiviral activities were dependent on cellular context. Importantly, we find that a significant fraction of compounds identified in Vero E6 cells also harbor antiviral activities in multiple human cell types and retain comparable potencies (**Figures 3a, 4a-b, ED6c and ED7**). Thus, we conclude that although the use of Vero E6 cells in the initial screening assay may preclude the identification of certain potential antivirals, most known drugs identified in this campaign disrupt viral replication independent of cellular background.

Of note, apilimod elicited some cytotoxic and/or cytostatic effects in *in vitro* cultivated cell lines at doses >100 nM (**Figure ED6a, 3b and 4a-b**). No cytotoxicity was observed in human iPSC-derived pneumocyte-like cells (**Figure ED9c**). However, even in cells presenting cytotoxic and/or cytostatic effects, we observed nearly maximal inhibition of viral replication at 100 nM, and the selectivity index (CC_{50}/EC_{50}) of the compound was determined to be 108, and thus we conclude that the observed impact on cellular viability or growth is independent of its antiviral activity.

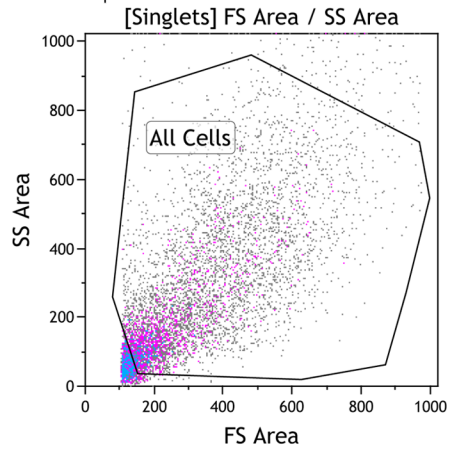
Supplementary Figure S1

H9 II-DMSO-2020-05-26-120347-16

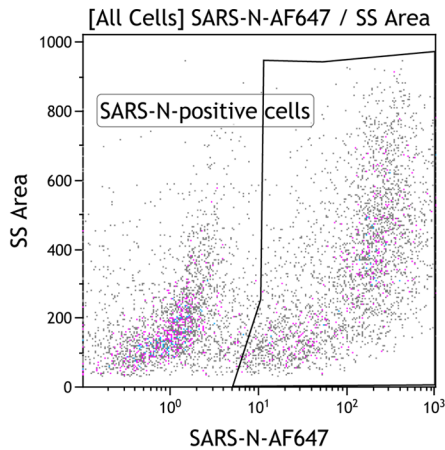
New Protocol 1 - Acquisition



Gate	Number	%Gated
All	40,361	100.00
Singlets	6,938	17.19



Gate	Number	%Gated
All	6,938	100.00
All Cells	5,624	81.06



Gate	Number	%Gated
All	5,624	100.00
SARS-N-positive cells	3,020	53.70

Supplementary References

- 54 Mootha, V. K. *et al.* PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics* **34**, 267-273, doi:10.1038/ng1180 (2003).
- 55 Butler, D. J. *et al.* Host, Viral, and Environmental Transcriptome Profiles of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *bioRxiv*, 2020.2004.2020.048066, doi:10.1101/2020.04.20.048066 (2020).
- 56 Deprez, M. *et al.* A single-cell atlas of the human healthy airways. *bioRxiv*, 2019.2012.2021.884759, doi:10.1101/2019.12.21.884759 (2019).
- 57 Kwan, C. Y. & Achike, F. I. Tetrandrine and related bis-benzylisoquinoline alkaloids from medicinal herbs: cardiovascular effects and mechanisms of action. *Acta pharmacologica Sinica* **23**, 1057-1068 (2002).
- 58 Sakurai, Y. *et al.* Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. *Science* **347**, 995-998, doi:10.1126/science.1258758 (2015).
- 59 Inciardi, R. M. *et al.* Cardiac Involvement in a Patient With Coronavirus Disease 2019 (COVID-19). *JAMA cardiology*, doi:10.1001/jamacardio.2020.1096 (2020).
- 60 Kapoor, A. *et al.* Cardiovascular risks of hydroxychloroquine in treatment and prophylaxis of COVID-19 patients: A scientific statement from the Indian Heart Rhythm Society. *Indian Pacing and Electrophysiology Journal*, doi:https://doi.org/10.1016/j.ipej.2020.04.003 (2020).
- 61 D'Alessandro, S. *et al.* The Use of Antimalarial Drugs against Viral Infection. *Microorganisms* **8**, doi:10.3390/microorganisms8010085 (2020).
- 62 Lucile White, E. *et al.* A TIBO derivative, R82913, is a potent inhibitor of HIV-1 reverse transcriptase with heteropolymer templates. *Antiviral Research* **16**, 257-266, doi:https://doi.org/10.1016/0166-3542(91)90005-C (1991).
- 63 De Wit, S. *et al.* Pharmacokinetics of R 82913 in AIDS patients: a phase I dose-finding study of oral administration compared with intravenous infusion. *Antimicrobial Agents and Chemotherapy* **36**, 2661-2663, doi:10.1128/aac.36.12.2661 (1992).

- 64 Garrelts, J. C. Clofazimine: a review of its use in leprosy and Mycobacterium avium complex infection. *DICP* **25**, 525-531, doi:10.1177/106002809102500513 (1991).
- 65 Bjorck, L., Grubb, A. & Kjellen, L. Cystatin C, a human proteinase inhibitor, blocks replication of herpes simplex virus. *Journal of virology* **64**, 941-943 (1990).
- 66 Zhou, Y. & Simmons, G. Development of novel entry inhibitors targeting emerging viruses. *Expert Rev Anti Infect Ther* **10**, 1129-1138, doi:10.1586/eri.12.104 (2012).
- 67 Mori, Y. *et al.* Processing of Capsid Protein by Cathepsin L Plays a Crucial Role in Replication of Japanese Encephalitis Virus in Neural and Macrophage Cells. *Journal of virology* **81**, 8477-8487, doi:10.1128/jvi.00477-07 (2007).
- 68 Simmons, G. *et al.* Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11876-11881, doi:10.1073/pnas.0505577102 (2005).
- 69 Marzi, A., Reinheckel, T. & Feldmann, H. Cathepsin B & L Are Not Required for Ebola Virus Replication. *PLOS Neglected Tropical Diseases* **6**, e1923, doi:10.1371/journal.pntd.0001923 (2012).
- 70 Eastell, R. *et al.* Safety and efficacy of the cathepsin K inhibitor ONO-5334 in postmenopausal osteoporosis: the OCEAN study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **26**, 1303-1312, doi:10.1002/jbmr.341 (2011).
- 71 Eastell, R. *et al.* Effect of ONO-5334 on bone mineral density and biochemical markers of bone turnover in postmenopausal osteoporosis: 2-year results from the OCEAN study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **29**, 458-466, doi:10.1002/jbmr.2047 (2014).
- 72 Rutherford, A. C. *et al.* The mammalian phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) regulates endosome-to-TGN retrograde transport. *J Cell Sci* **119**, 3944-3957, doi:10.1242/jcs.03153 (2006).
- 73 Gayle, S. *et al.* Identification of apilimod as a first-in-class PIKfyve kinase inhibitor for treatment of B-cell non-Hodgkin lymphoma. *Blood* **129**, 1768-1778, doi:10.1182/blood-2016-09-736892 (2017).

- 74 Sultana, F. *et al.* Snx10 and PIKfyve are required for lysosome formation in osteoclasts. *Journal of cellular biochemistry* **121**, 2927-2937, doi:10.1002/jcb.29534 (2020).
- 75 Qiu, S. *et al.* Ebola virus requires phosphatidylinositol (3,5) bisphosphate production for efficient viral entry. *Virology* **513**, 17-28, doi:https://doi.org/10.1016/j.virol.2017.09.028 (2018).
- 76 Food_And_Drug_Administration. Coronavirus (COVID-19) Update: FDA Issues Emergency Use Authorization for Potential COVID-19 Treatment. (2020).
- 77 Matthew, A. N., Kurt Yilmaz, N. & Schiffer, C. A. Mavyret: A Pan-Genotypic Combination Therapy for the Treatment of Hepatitis C Infection Published as part of the Biochemistry series "Biochemistry to Bedside". *Biochemistry* **57**, 481-482, doi:10.1021/acs.biochem.7b01160 (2018).
- 78 Ferenci, P. New anti-HCV drug combinations: who will benefit? *The Lancet. Infectious diseases* **17**, 1008-1009, doi:10.1016/S1473-3099(17)30486-3 (2017).
- 79 Cihlar, T. & Fordyce, M. Current status and prospects of HIV treatment. *Current opinion in virology* **18**, 50-56, doi:10.1016/j.coviro.2016.03.004 (2016).
- 80 Schaad-Lanyi, Z., Dieterle, W., Dubois, J. P., Theobald, W. & Vischer, W. Pharmacokinetics of clofazimine in healthy volunteers. *Int J Lepr Other Mycobact Dis* **55**, 9-15 (1987).
- 81 Clofazimine. *Tuberculosis (Edinb)* **88**, 96-99, doi:10.1016/s1472-9792(08)70006-4 (2008).
- 82 Taglialatela, M. *et al.* Molecular basis for the lack of HERG K⁺ channel block-related cardiotoxicity by the H1 receptor blocker cetirizine compared with other second-generation antihistamines. *Mol Pharmacol* **54**, 113-121, doi:10.1124/mol.54.1.113 (1998).
- 83 Kumar, M. *et al.* Multistage Antiplasmodium Activity of Astemizole Analogues and Inhibition of Hemozoin Formation as a Contributor to Their Mode of Action. *ACS Infect Dis* **5**, 303-315, doi:10.1021/acsinfecdis.8b00272 (2019).
- 84 Cao, Y. C., Deng, Q. X. & Dai, S. X. Remdesivir for severe acute respiratory syndrome coronavirus 2 causing COVID-19: An evaluation of the evidence. *Travel Med Infect Dis*, 101647, doi:10.1016/j.tmaid.2020.101647 (2020).

- 85 Dworetzky, S. I. *et al.* Phenotypic Alteration of a Human BK (hSlo) Channel by hSlo β Subunit Coexpression: Changes in Blocker Sensitivity, Activation/Relaxation and Inactivation Kinetics, and Protein Kinase A Modulation. *The Journal of Neuroscience* **16**, 4543-4550, doi:10.1523/jneurosci.16-15-04543.1996 (1996).
- 86 Nelson, E. A. *et al.* The phosphatidylinositol-3-phosphate 5-kinase inhibitor apilimod blocks filoviral entry and infection. *PLoS Negl Trop Dis* **11**, e0005540, doi:10.1371/journal.pntd.0005540 (2017).
- 87 Vallet, S. *et al.* MLN3897, a novel CCR1 inhibitor, impairs osteoclastogenesis and inhibits the interaction of multiple myeloma cells and osteoclasts. *Blood* **110**, 3744-3752, doi:10.1182/blood-2007-05-093294 (2007).
- 88 Vergunst, C. E. *et al.* MLN3897 plus methotrexate in patients with rheumatoid arthritis: safety, efficacy, pharmacokinetics, and pharmacodynamics of an oral CCR1 antagonist in a phase IIa, double-blind, placebo-controlled, randomized, proof-of-concept study. *Arthritis Rheum* **60**, 3572-3581, doi:10.1002/art.24978 (2009).
- 89 Ochi, Y. *et al.* Effects of ONO-5334, a novel orally-active inhibitor of cathepsin K, on bone metabolism. *Bone* **49**, 1351-1356, doi:10.1016/j.bone.2011.09.041 (2011).
- 90 Tanaka, M., Hashimoto, Y., Hasegawa, C., Deacon, S. & Eastell, R. Antiresorptive effect of a cathepsin K inhibitor ONO-5334 and its relationship to BMD increase in a phase II trial for postmenopausal osteoporosis. *BMC Musculoskelet Disord* **18**, 267, doi:10.1186/s12891-017-1625-y (2017).
- 91 Brunton, V. G. & Workman, P. In vitro antitumour activity of the novel imidazoisquinoline SDZ 62-434. *Br J Cancer* **67**, 989-995, doi:10.1038/bjc.1993.181 (1993).
- 92 Shinozuka, T. *et al.* Discovery of DS-6930, a potent selective PPAR γ modulator. Part II: Lead optimization. *Bioorganic & Medicinal Chemistry* **26**, 5099-5117, doi:<https://doi.org/10.1016/j.bmc.2018.09.005> (2018).
- 93 O'Neill, P. M. *et al.* Candidate Selection and Preclinical Evaluation of N-tert-Butyl Isoquine (GSK369796), An Affordable and Effective 4-Aminoquinoline Antimalarial

- for the 21st Century. *Journal of Medicinal Chemistry* **52**, 1408-1415, doi:10.1021/jm8012618 (2009).
- 94 GSK. Single-Blind, Placebo-Controlled, Randomized Study Testing Single Ascending Doses Of GSK369796 In Healthy Subjects.
 - 95 De Wit, S. *et al.* Pharmacokinetics of R 82913 in AIDS patients: a phase I dose-finding study of oral administration compared with intravenous infusion. *Antimicrob Agents Chemother* **36**, 2661-2663, doi:10.1128/aac.36.12.2661 (1992).
 - 96 Lucile White, E. *et al.* A TIBO derivative, R82913, is a potent inhibitor of HIV-1 reverse transcriptase with heteropolymer templates. *Antiviral Research* **16**, 257-266, doi:[https://doi.org/10.1016/0166-3542\(91\)90005-C](https://doi.org/10.1016/0166-3542(91)90005-C) (1991).
 - 97 Pedersen, J. Z. & Finazzi-Agrò, A. Protein-radical enzymes. *FEBS Letters* **325**, 53-58, doi:10.1016/0014-5793(93)81412-s (1993).
 - 98 Ognyanov, V. I. *et al.* Design of potent, orally available antagonists of the transient receptor potential vanilloid 1. Structure-activity relationships of 2-piperazin-1-yl-1H-benzimidazoles. *J Med Chem* **49**, 3719-3742, doi:10.1021/jm060065y (2006).
 - 99 Lazar, J., Gharat, L., Khairathkar-Joshi, N., Blumberg, P. M. & Szallasi, A. Screening TRPV1 antagonists for the treatment of pain: lessons learned over a decade. *Expert Opin Drug Discov* **4**, 159-180, doi:10.1517/17460440802681300 (2009).
 - 100 Uchii, M., Takashima, M., Sugiyama, T. & Kosaka, N. Effect of KW-8232 on bone turnover in ovariectomized rats. *Folia Pharmacologica Japonica* **112**, 315-321, doi:10.1254/fpj.112.315 (1998).
 - 101 Uchii, M., Takashima, M., Sugiyama, T. & Kosaka, N. Effect of KW-8232, a novel anti-osteoporotic agent, on bone loss in sciatic neurectomized rats. *Jpn J Pharmacol* **78**, 241-243, doi:10.1254/jjp.78.241 (1998).
 - 102 Hoffmann, M. *et al.* Analysis of Resistance of Ebola Virus Glycoprotein-Driven Entry Against MDL28170, An Inhibitor of Cysteine Cathepsins. *Pathogens* **8**, doi:10.3390/pathogens8040192 (2019).
 - 103 Scott, C. *et al.* SB-616234-A (1-[6-(cis-3,5-dimethylpiperazin-1-yl)-2,3-dihydro-5-methoxyindol-1-yl]-1-[2'meth yl-4'-(5-methyl-1,2,3-oxadiazol-3-yl)biphenyl-4-yl]methanone hydrochloride): a novel, potent and selective 5-HT_{1B} receptor

- antagonist. *Neuropharmacology* **50**, 984-990,
doi:10.1016/j.neuropharm.2006.01.008 (2006).
- 104 Casero, R. A., Jr. & Woster, P. M. Recent advances in the development of
polyamine analogues as antitumor agents. *J Med Chem* **52**, 4551-4573,
doi:10.1021/jm900187v (2009).
- 105 Bjorck, L. *et al.* Bacterial growth blocked by a synthetic peptide based on the
structure of a human proteinase inhibitor. *Nature* **337**, 385-386,
doi:10.1038/337385a0 (1989).