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Dear *Nature Microbiology* Editorial Board,

We are writing to submit the manuscript "Quantifying the effects of single mutations on viral escape from broad and narrow antibodies to an H1 influenza hemagglutinin" for consideration as an Article at *Nature Microbiology*.

The discovery of broadly neutralizing antibodies against influenza virus has inspired hopes that it might be possible to design vaccines or therapeutics that are more resistant to viral escape. However, the fundamental conceptual basis for these approaches – the idea that the virus has an inherently more difficult time escaping from some antibodies – has never been formally tested. The primary reason is that existing approaches to characterize viral antibody escape do not provide comprehensive quantitative data that can be compared across antibodies in a meaningful way.

Here we completely experimentally map how *all* single amino-acid mutations to influenza virus hemagglutinin affect neutralization by both broadly neutralizing and narrow strain-specific antibodies. We use a novel computational framework to quantify the antigenic effects of all mutations in a way that can be directly compared across antibodies.

We show that there are mutations that enhance influenza virus's resistance to even the broadest antibodies. However, the magnitudes of these antigenic effects vary dramatically across antibodies. The virus can escape some broad antibodies very easily, but it can only modestly shift its sensitivity to others. Our work therefore provides the first rigorous comparison of the ease of viral escape from different antibodies, and so will be of value in guiding efforts to better control influenza. Our work also is the first characterization of mutations that mediate escape (albeit weakly) from some of the broadest anti-influenza antibodies.

We expect that our manuscript will be interest to virologists, evolutionary biologists studying viruses, and immunologists interested in antibodies. Therefore, we think that the manuscript is appropriate for a broad journal such as *Nature Microbiology*.

Our paper touches on influenza virus, antibodies, evolutionary biology, computational biology, and deep sequencing. We have therefore suggested reviewers with expertise in each of these areas:

- **Nick Heaton** (Duke, https://sites.duke.edu/heatonlab/) is an expert on influenza virus and its mutational tolerance. His e-mail is: nicholas.heaton@duke.edu
- **George Georgiou** (University of Texas, https://georgiou.icmb.utexas.edu/) is an expert on anti-viral antibodies, including those against influenza virus. His e-mail is: gg@che.utexas.edu
- **Sarah Cobey** (University of Chicago, https://cobeylab.uchicago.edu/) is an expert in computational approaches to study viral evolution and immune escape. Her e-mail is: cobey@uchicago.edu
- **Adam Lauring** (University of Michigan, https://sites.google.com/a/umich.edu/the-lauring-lab/) is an expert on the study of influenza virus using deep sequencing. His e-mail is: alauring@med.umich.edu
- **Colin Russell** (AMC Amsterdam, https://www.whocc.infectiousdisease.cam.ac.uk/directory/car44@cam.ac.uk) is an expert in influenza virus evolution. His e-mail is: car44@cam.ac.uk
- **Claus Wilke** (University of Texas, http://wilkelab.org/) is an expert in computational studies of virus evolution. His e-mail is: wilke@austin.utexas.edu
- **Ian Wilson** (Scripps, https://wilson.scripps.edu/) is an expert on hemagglutinin structure and anti-influenza antibodies. His e-mail is: wilson@scripps.edu
- **James Crowe** (Vanderbilt, https://www.vumc.org/crowe-lab/) is an expert on anti-influenza antibodies. His e-mail is: james.crowe@vanderbilt.edu

Finally, we mention a few considerations related to the Supplementary Information for our manuscript. Our study involves the computational analysis of large deep-sequencing datasets. A major strength of our manuscript is that we have written a completely reproducible computational pipeline that performs all the data analysis and generates many of the figures. Inclusion of this pipeline is an important part of our manuscript, because we expect many other groups will want to build on our study. However, this pipeline does not fit in a standard file format, so we have included it in a ZIP file as one of the supplementary files (File S3). In addition, we have several other large supplementary figures and files that are important to fully represent the data and enable our work to be reproduced. We hope that *Nature Microbiology* can accommodate these figures and files in the interest of open and transparent science.

Thanks for your time and consideration.

Sincerely,

Jesse D. Bloom, Ph.D.