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Dear *eLife* Editorial Board,

We are writing to submit the full version of our manuscript, “Extreme heterogeneity of influenza virus infection in single cells.” This manuscript has already passed the initial editorial screening.

We note that Steve Quake’s group has told us that they also plan to soon submit a paper to *eLife* on single-cell sequencing of flavivirus infected cells. That manuscript is complementary to our own, as it also provides a single-cell view of what happens to cells during viral infection. If both papers are deemed appropriate for *eLife*, we hope that they might be published together!

In our paper, we look at what happens during the infection of single cells by influenza virus. It is known that such infection leads to profound transcriptional changes, as the virus hijacks the cell to pump out viral mRNAs, and as the cell attempts to mount innate-immune responses that inhibit the infection. However, to date, the transcriptional dynamics of viral infection have been studied mostly by bulk analysis of many infected cells.

Here we use single-cell mRNA sequencing to quantify all cellular and viral transcripts during influenza virus infection. We find extreme heterogeneity among nominally identical cells infected with the same stock of virus. Most infected cells express only a modest amount of viral mRNA – but some cells derive over half their mRNA from virus at later time points. Our results therefore demonstrate remarkable divergence in the outcome of infection among cells.

Because of the comprehensive nature of our data, we can examine the viral and cellular factors that underpin this heterogeneity. We show that the absence of expression of some viral genes in some cells is a significant contributing factor. We also identify host genes that co-vary with viral transcriptional load. Finally, we show that activation of the innate-immune response is very rare, even when the virus fails to express its primary antagonist of these pathways. We also make a wealth of other important observations, including about the role that co-infection can play when some virions fail to express the full complement of viral genes.

Our work is the first holistic examination of the transcriptional outcome of viral infection in single cells, and so will be of broad interest in the field of single-cell studies, to virologists, and to immunologists. In particular, our work opens the door to understanding how heterogeneity among cells shapes the bulk outcomes of virus infection.

We suggest **Aviv Regev**, **Arup Chakraborty**, and **Patricia Wittkopp** as Senior Editors for our manuscript.

We suggest **Richard Neher**, **Aleksandra Walczak**, **Michael Laub**, and **Stephen Goff** as Reviewing Editors.

We ask that our paper be evaluated by reviewers with expertise in single-cell studies, virology, and computational biology. The following individuals would be well qualified to evaluate our work:

- **Rahul Satija** (New York Genome Center, <http://satijalab.org/>, rsatija@nygenome.org) is an expert on single-cell studies, and has performed pioneering work on innate-immune responses in single cells.
- **Alex Shalek** (MIT, <http://shalek.wpengine.com/>, shalek@mit.edu) is an expert on single-cell studies, and has performed pioneering work on innate immunity in single cells.
- **Long Cai** (Caltech, <http://singlecell.caltech.edu/cailab/>, lcai@caltech.edu) is an expert on single-cell systems biology.
- **Adam Lauring** (University of Michigan, <https://sites.google.com/a/umich.edu/the-lauring-lab/>, alauring@med.umich.edu) is an expert on influenza virus and its study via deep sequencing.
- **Seema Lakdawala** (University of Pittsburgh, <http://www.mmg.pitt.edu/lab/lakdawala-lab>, lakdawala@pitt.edu) is an expert on influenza virus.
- **Chris Brooke** (University of Illinois, <https://www.brookelab.org/>, cbrooke@illinois.edu) is an expert on influenza virus and viral defective particles.
- **Katia Koelle** (Emory University, <http://www.biology.emory.edu/index.cfm?faculty=471>, katia.koelle@emory.edu) is an expert on the within-host dynamics of influenza virus.
- **Ben TenOever** (Mt. Sinai, <http://labs.icahn.mssm.edu/tenoeverlab/>, Benjamin.tenOever@mssm.edu) is an expert on influenza virology.

Thanks for your time and consideration.

Sincerely,



Jesse D. Bloom, Ph.D.