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Dear *eLife* Editorial Board,

I am writing to submit our manuscript "Single-cell virus sequencing of influenza infections that trigger innate immunity" for consideration as a Research Advance in *eLife*.

In this manuscript, we take a major step towards elucidating what factors determine if the immune system recognizes viral infection. It has been known for some time that infection with influenza virus only rarely activates innate immunity in single cells, and our own recent work in *eLife* (7:e32303) and follow-up studies by others have shown that this is just one form of the extreme cell-to-cell heterogeneity that characterizes viral infection.

However, it remains unclear *why* only some cells detect viral infection, and why the outcome of infection is so variable from cell to cell. A major reason for this gap in knowledge is that current single-cell techniques (e.g., single-cell RNA-seq, flow cytometry) have a limitation: they count the abundance of transcripts or proteins, but do not reveal if they have mutations.

We overcome this limitation by developing a new method to simultaneously determine the full transcriptome and the sequences of all viral genes in single influenza-infected cells. We then show that this methodological advance is extremely important for understanding heterogeneity in viral infection, particularly with respect to induction of innate immunity.

Specifically, we show that over three-quarters of infected cells that activate an innate immune response are actually infected with virions that have a mutation or some other genetic defect. We identify four classes of viral defects that we validate increase the frequency of innate-immune detection. Overall, our work shows that viral genetic variation is a major contributor to the heterogeneity in the outcome of infection.

Our work will be of interest to computational biologists, virologists, immunologists, and scientists interested in new single-cell techniques. From a purely methodological standpoint, it is the first study to determine the actual sequences of the virions infecting single cells—and from a biological standpoint, it shows that knowing these sequences is essential to understanding why infection proceeds in the way that it does.

We are submitting this study as a Research Advance on our earlier *eLife* study (7:e32303), which used single-cell RNA-seq to characterize the transcriptomes of influenza-infected cells without virus sequence information. Our earlier study posed the question (answered by our new study) of why innate-immune detection is so rare. We note that our earlier study has already received wide interest: it was discussed on the This Week In Virology podcast, and has been cited over a dozen times in the six months since its publication. We think the current study is likely to be of even greater interest, as it answers key biological questions about how viral mutations contribute to cell-to-cell heterogeneity. Since our new study builds on many of the methods in the earlier study, we think it would be appropriate for them to be linked as the Research Advance format allows.

Finally, we note that consistent with *eLife*'s emphasis on open access and reproducibility, we have created a public GitHub repository that contains all the computer code, data analysis, and the paper itself: https://github.com/jbloomlab/IFNsorted_flu_single_cell

We hope that this our manuscript can be handled by the editors and reviewers of the original paper. But if that is not possible, we have also listed editor and reviewer suggestions below:

Senior editor:
- **Aviv Regev**: expert in single-cell techniques and immunology.
- **Arup Chakraborty**: expert in computational biology and viral immunology.

Reviewing editors:
- **Richard Neher**: expert in virology and computational biology
- **Aleksandra Walczak**: expert in virology and computational biology
- **Stephen Goff**: expert in virology and anti-viral immunity
- **Karla Kirkegaard**: expert in virology

Reviewers: 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 studied immune responses in single cells.
- **Alex Shalek** (MIT, http://shalek.wpengine.com/, shalek@mit.edu) is an expert on single-cell studies, and has studied immune responses 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.