Dear Lynden,

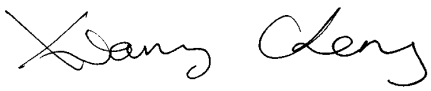
Thank you for reviewing our manuscript "Density Fluctuations and Energy Spectra of 3D Bacterial Suspensions" (Ms. # abi8621) and sending us the reviewers' reports.

We are very pleased to see the positive comments from both reviewers. Reviewer 1 commented that our study "contains a novel study of the coupling and universality of the density-energy spectra pairing". The reviewer found our work "interesting and enjoyed reading the paper." Reviewer 2 also commented that our manuscript shows interesting experimental results.

Both reviewers raised important technical concerns, which we have satisfactorily addressed in the revision. Reviewer 1 further commented that the original writing of our manuscript is too technical to appeal a wider audience and suggested us to simplify the technical discussions of our writing. Following the suggestion of the reviewer, we have first substantially revised the introduction of the manuscript, which now introduces density fluctuations—the main theme of our study—in a much more accessible way (please see the first paragraph of the revised manuscript). In addition, we have also thoroughly revised the main body of the manuscript and removed all the detailed calculations that are not directly relevant to the main conclusion of our manuscript. After the revision, we have asked several researchers in the field of condensed matter physics, microbiology, chemical engineering and materials science at University of Minnesota and ESPCI Paris to help us review the revised manuscript and have consistently received very positive comments on the accessibility of our revised manuscript. Hence, we believe that the revised manuscript is now approachable and appealing to the broad technical audience of *Science Advances*.

Below we shall address the comments/suggestions of the reviewers point-by-point. We hope that the positive comments from the reviewers and our detailed responses to the reviewers' questions will convince you that our revised manuscript is now suitable for publication in *Science Advances*.

Sincerely yours,



Xiang Cheng

On behalf of all of the co-authors

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We report below a detailed response to the referees' reports. The original referee comments are in *italic*, and our response appears in normal font. When addressing the referees' concerns, we also explicitly listed all the detailed changes we have made in the revised manuscript.

Response to Reviewer 1:

We thank the reviewer for the very generous positive comments on our work. We also appreciate the reviewer for the constructive suggestions, which improve not only the technical strength of our manuscript but also its readability for the general technical audience of *Science Advance*. Following the comments/suggestions of the Reviewer, we have thoroughly revised the manuscript, which is now much more approachable to scientists and engineers outside the immediate field of active matter. Below, we shall address the comments/suggestions of the reviewer point-by-point:

1. pg. 1 Introduction My Impression of the Intro is that it contains useful information, but I would appreciate a paragraph at the beginning zooming out a bit before diving deep into the methods and exploration of this paper. Possible more on observations of these phenomena using plain language. I think this would make the paper more appealing/accessible to a wider audience.

Following the suggestion of the reviewer, we have substantially rewritten and simplified the introduction of the manuscript. In particular, we added a new paragraph at the beginning to introduce the general concept of density fluctuations in equilibrium systems, which allows us to frame our work in a broader context that should be familiar to any audience who has received basic training in thermodynamics and statistical mechanics. After the general introduction, we then introduced the concept of active matter and giant number fluctuations—the central theme of our study—in more accessible simple language. Furthermore, we have removed the technical discussion of the energy spectra and active turbulence from the Introduction, which we believe distract the main theme of our manuscript on density fluctuations. Lastly, we have also rewritten the summary of the main results of our study in plainer language in the introduction.

In addition to the Introduction, we have also thoroughly revised the entire manuscript and removed all the detailed technical discussions and calculations that are not directly relevant to the main conclusion of our manuscript. Particularly, we have removed the lengthy theoretical discussion of the universal power-law exponent of energy spectra, as well as the tentative scaling argument for the density fluctuations–energy spectra coupling, which not only made the discussion of the original manuscript overly technical but also distracted the main point of the manuscript. Finally, we agree with the reviewer that movies are probably the best way to appeal the interest of general audience. Hence, we have provided an additional supplementary video, which shows the steady-state active turbulence with strong density fluctuations in high-concentration bacteria suspensions (the new SM video 1). The video provides a direct visual illustration of strong density fluctuations as well as the flow of active turbulence in dense bacterial suspensions—the two key phenomena explored in our study.

After the revision, we have presented the revised manuscript (including the supplementary videos) to several researchers in the field of condensed matter physics, microbiology, chemical engineering and materials science at University of Minnesota and ESPCI Paris and have consistently received very positive comments on the accessibility of the manuscript. Hence, thanks to the suggestion of the reviewer, we believe that the revised manuscript with the new introduction and supplementary videos is appealing to the broad technical audience targeted by *Science Advances*. Our results should be of general interests to not only microbiologists and biophysicists studying the collective swimming of bacteria, but also physicists and material scientists studying soft materials, active matter and nonequilibrium statistical mechanics.

Lastly, we'd like to stress that, as pointed out by the reviewer, our manuscript reported the first experimental study on density fluctuations in 3D bacterial suspensions. Our study provided a unifying approach to understand the emergent collective dynamics of dense bacterial suspensions. The results reported in this manuscript represented the most important findings of our group out of our intensive research efforts on active fluids over the last 7 years. Indeed, the figures from the current manuscript, which we have posted on arXiv while submitting this manuscript, have already been cited and discussed by the very recent review summarizing the key progress in the study of active turbulence over the last two decades (see Fig. 1d in "Active Turbulence" by Alert et al., arXiv:2104.02122 and the associated discussions). Thus, we believe the scope and quality of the work meet the high standard of *Science Advances*. The manuscript is a better fit for *Science Advances* than for other more specialized journals.

2. pg. 3 You want to study 3D motion, but the experimental domain is 20mm × 3mm × 140μm. One could argue that the depth is very small compared to the other two dimensions making it appear as more of a quasi-2d suspension than true 3d. I understand you can still measure things in the z direction, but an explanation or discussion about why these observations would be the same as a true bulk suspension where all dimensions are similar 1mm × 1mm × 1mm may make the arguments following this point stronger.

Thanks the reviewer for raising this important question, which was missing in the discussion of our original manuscript. It is certainly important to justify why our system is a 3D bulk system, instead of a 2D or quasi-2D system similar to previous studies.

First of all, the smallest dimension of 140 μm is more than two orders of magnitude larger than the size of single bacteria on the order of 1 μm. In sharp contrast, previous studies on the density fluctuations of active fluids all focused on 2D or quasi-2D systems, where a single layer of active particles were investigated (e.g. a monolayer of bacteria or motile eukaryotic cells on the surface of agar plates (Ref. 14, 15, 17), and a monolayer of vibrated granular rods (Ref. 10-12)).

Second, with the minimal dimension of 140 μm, the volume of our samples is on the order of several microliters, which contains more than 10^7 bacteria at low $\phi = 0.018$. Simulations and kinetic theories have already shown that the dynamics of bacterial suspensions reach the bulk limit at such a large scale (Ref. 36). Our previous experiments have also demonstrated that the relation between the size of turbulent vortex and the system size stabilizes at 140 μm, reaching the bulk

limit (Ref. 37). More recent experiments further confirmed our finding and showed that the onset of collective motions is insensitive to the system size above ~ 140 μm (Ref. 38). All these numerical and experimental works have shown that the dynamics of bacterial suspensions have already reached the bulk limit at 140 μm and would not change if the dimension increases further.

Third, the dynamics of bulk bacterial suspensions are isotropic away from system boundaries. Thus, imaging a 2D plane within a bulk sample away boundaries yields representative results and provides all the necessary statistical information to characterize the flow structures and density fluctuations of the 3D system. Imaging any other planes at different z would give statistically the same results, as long as the z position of the planes is sufficiently away from boundaries.

To clarify this important point, we have added a new paragraph in the "Experiments" section of the revised manuscript (the second paragraph of the section):

"Note that the smallest dimension of our system is H = 140 μm, which is two orders of magnitude larger than the size of single bacteria. Recent numerical and experimental studies have shown that the dynamics of bacterial suspensions exhibit bulk behaviors at such a large scale (*36-38*). Away from system boundaries, the flow of bacterial suspensions is isotropic. Hence, imaging a 2D plane within a bulk suspension yields representative results and provides all the necessary statistical information on the density fluctuations and flow structures of bulk 3D suspensions."

3. pg. 4 The findings suggest active turbulence occurs at the critical volume fraction ϕ_c = .04 and a reference is given. Is this large enough because typically a suspensions is assumed to be essentially dilute at 5% or less and the transition to collective swimming occurs around 20 – 25% volume fraction. So the ϕ_c for active turbulence here seems small and a comment on if this is do to collective swimming or not would be useful.

The seemingly low transition concentration arises due to the long-range nature of hydrodynamic interaction between bacteria, which is the leading factor driving the collective swimming of bacteria. Since the dipolar flow field of bacteria decays slowly with distance following $1/r^2$ (Ref. 56), the hydrodynamic interaction is very long-ranged. Thus, the collective swimming promoted by this long-range interaction occurs as very low concentrations.

We have experimentally measured the transition concentration as a function of both the swimming speed of bacteria and the fraction of active swimmers in mixtures of mobile and immobile bacteria in our previous study (Ref. 41). The transition concentration in the current study quantitatively agrees with the results of our previous study. The value also quantitatively agrees with the prediction of the kinetic theory of active suspensions (Ref. 47),

$$\phi_c = \frac{\eta V_b}{\xi l}\left[\frac{5}{\tau_t} + 30D_r\right]\frac{1}{v}. \quad \text{(R1)}$$

Here, η = 1 mPa·s is the viscosity of the buffer. $V_b \approx 2.20$ μm^3 is the volume of bacteria. $\xi = 1.28 \times 10^{-8}$ kg/s is the drag coefficient of bacterial body orientated along its major axis. l = 1.9 μm is the distance of the force dipole. τ_t = 1 s is the inverse tumbling rate of bacteria. $D_r = 0.057$ s^{-1} is the effective diffusion coefficient of bacteria. At the average swimming speed of v = 15 μm/s for

bacteria in our suspensions, Equation (R1) predicts ϕ_c = 4.05%, quantitatively matching our experimental finding.

Since the focus of our current study is the density fluctuations of bacterial suspensions, instead of the transition concentration towards collective swimming, we refer the reviewer to our previous publication for the detailed information on the origin of the low transition concentration and its dependence on different physical parameters (Ref. 41).

To address the concern of the reviewer, we have added the following comment in pg. 5 of the revised manuscript:

"…, where ϕ_c is the transition concentration to active turbulence. ϕ_c = 4.05% in our experiments, quantitatively agreeing with our previous experiments as well as the prediction of kinetic theories (*41*)."

4. pg. 5 Concerning the density correlation time, is the reason we see a decrease in correlation time at large volume fraction past ϕ_c due to jamming? or maybe your concentrations were not high enough to investigate the jamming regime.

Even at the highest concentration explored in our study, the volume fraction of bacteria is still very low at ϕ < 10%, which is far away from the jamming point. On the contrary, rather than jamming, the average flow velocity of bacterial suspensions reaches maximum at high concentrations in active turbulence, resulting in low density correlation times at high ϕ. Note that a decrease in correlation time indicates an increase in system dynamics, instead of the arrest of dynamics at jamming. The new supplementary video (SM video 1) illustrates the strong active turbulent flow of dense bacterial suspensions at high ϕ. Practically, *E. coli* would die when the concentration is too high beyond our experimental regime. So it is not possible to explore the jamming transition with active *E. coli* suspensions.

5. pg. 7 You also say L = 140_m is a "large" system size, but it is small compared to the other dimensions. So do you mean large as in compared to the size of an individual bacterium.

Please see our answer to Question 2 above. Note that we have changed "*L*" to "*H*" in the revised manuscript to better represent the height of the chamber.

6. R7: pg. 15, in Figure 2E are the correlation lengths converging to a constant with density or constantly increasing. It is hard to tell in the image and a comment on which and why would be helpful.

Within experimental errors, both the density correlation length and the velocity correlation length reach constant at high concentrations. A small increase may exist. But this possible increasing

trend is certainly beyond the resolution of experiments. The result is indeed consistent with previous experiments on the velocity correlation of bacterial suspensions, where the velocity correlation length has been shown to reach a constant at high concentrations (Ref. 42). Here, our study provided the first experimental measurement on the density correlation length of bacterial suspensions and examined the similarities and differences between the velocity correlation and the density correlation.

Theoretically, the kinetic theories of active suspensions have predicted a long-wavelength hydrodynamic instability of the isotropic phase with random bacterial swimming, which results in the collective swimming of bacteria (Ref. 45–48). As a result, the length scale of the most unstable mode should be infinite in theory and is only constrained by the size of the system in practice. Hence, the velocity correlation length should be correlated with the size of the system, independent of bacterial concentrations above the transition concentration ϕ_c. Our previous experiments measuring the velocity correlation length in systems of different heights confirmed the prediction (Ref. 37), which showed the linear relation between the size of the system and the velocity correlation length, regardless bacterial concentrations. Density correlations lock to velocity correlations in concentrated bacterial suspensions with active turbulence due to the strong flow-density coupling demonstrated in our study. Thus, the density correlation length also approaches constant at high ϕ.

To address the concern, in the revised manuscript, we have further emphasized the converging trend (pg. 5): the density correlation length "reaches a *plateau* of ~ $5l_b$ above ϕ_c in high-ϕ suspensions with active turbulence" and the velocity correlation length "*saturates* above ϕ_c, consistent with previous experimental finding (*42*)".

In addition, we have explained our previous experimental finding regarding the constant velocity correlation length in the revised manuscript (pg. 5) as follows:

"Our previous study on the velocity correlation of high-concentration bacterial suspensions in a planar Couette cell with an adjustable height have shown that the velocity correlation length is linearly proportional to the size of the system (*37*), which is set by the minimum dimension of the sealed chamber at H = 140 μm in the current study."

Response to Reviewer 2:

We thank Reviewer 2 for carefully reading our manuscript and appreciate many insightful and constructive suggestions/comments from the reviewer. We have thoroughly addressed the concerns of the reviewer and revised our manuscript accordingly. The revision greatly improves the quality of our manuscript. Below, we shall address the comments of the reviewer in detail:

1. A major theme of the paper is that there are "anomalous density fluctuations", a word used in the abstract but not defined there. Later in the introduction it seems to be suggested that anomalous means the number of particles found in a volume grows faster than linearly with the volume (or mean number of particles) and I presume to be anomalous this should extend over all length scales from the bacterium size to a length of the order of the container size. However, the extent to which the paper backs up this claim (if I understand the claim correctly) is not very clear. After noting that the density fluctuations scale as a power law up to the density correlation length, the corroboration of the claim to anomalous seems to rely critically on the last sentence of p4 "The saturated density correlation length is ½ of the saturated velocity length, which in turn is linearly proportional to the system size (36)." So we need to assume that comparable lengths in this experimental report along with another study showing the velocity length is on the order of the system size means the density length is of order of the system size. And no details are provided or specific references made to understand what has been done in (36). Is the "size of the system" the channel thickness (since that is the smallest dimension). Is the study of (36) identical in all ways to the current study except that the channel thickness was changed in (36) and not in the present study?

We apologize for our insufficient account of the literature, which caused the confusion in the original manuscript as pointed out by the reviewer.

First of all, to avoid the ambiguity associated with the term "anomalous density fluctuations" pointed out by the reviewer, we have modified the abstract, where we have now changed "anomalous density flucatuions" to "giant number fluctuations", a term that has been widely used and well defined in the literature to describe density fluctuations in active fluids (see e.g. Ref. 10-20 and 22-32).

Kinetic theories of active suspensions predicted a long-wavelength hydrodynamic instability of the isotropic state of random bacterial swimming at wavenumber $q \rightarrow 0$ above a critical bacterial concentration, which results in the collective swimming and active turbulence of bacteria (Ref. 45–48). Thus, the length scale of the most unstable mode should be infinite in theory and is only constrained by the size of the system in practice. In our case, the system size is the smallest dimension set by the height of the sealed chamber ($H = 140$ μm). Our previous study measured the velocity correlations of high-concentration bacterial suspensions with collective swimming in systems of different heights [Ref. 37 (Ref. 36 in the original manuscript)], where we showed that the velocity correlation length increases linearly with the size of system (Fig. 6D of Ref. 37). The geometry of our previous study is similar to the one adapted in the current study, where the size of the system is set by the height, i.e. its minimum dimension, while the other two dimensions are

much larger (please see our answer to the next question for more details). Our finding has been further confirmed by the recent study of Martinez et al. (Ref. 38). Eric Clement from ESPCI also showed unpublished results on the linear relation between the velocity correlation length and the size of systems over a much large range in a talk last year.

To eliminate the confusion, we have explained the geometry and the results of our previous study and clarified the meaning of the size of the system in the revised manuscript (pg. 5).

"Our previous study on high-ϕ bacterial suspensions in a planar Couette cell with an adjustable height have shown that the velocity correlation length is linearly proportional to the size of the system (*37*), which is set by the minimum dimension of the sealed chamber H = 140 μm in our current study."

2. This seems to be at least the third paper by the current group on 3D bacteria measurements that are similar. Ref (44), (36) and the present submission. Are these all based on the same set of experiments but with different pieces of the measurements reported? Or are they different in some ways? The similarities and differences are important to judge what can be learned from the other studies and what is the significance that justifies the new paper and this should be discussed in the introduction.

Indeed, we have published two papers previously on the dynamics of bacterial suspensions over the last five years. Although we had to cite our own published works for results previously obtained in order to avoid repeating the same experiments, the focus and the scope of our current work are very different from these previous studies.

In Ref. 37 (the original Ref. 36) titled "Symmetric shear banding and swarming vortices in bacterial superfluids", we studied the rheological response, particularly the shear banding profile, of bacterial suspensions under shear. We used a planar Couette shear cell to apply controlled shear to high-concentration bacterial suspensions and measured the resulting shear profiles within the suspensions. The custom shear cell we constructed has two fixed dimensions of 2.5 mm by 2.5 mm and an adjustable height H, which can be varied from 5 μm up to 160 μm. Thanks to the adjustable height, we were able to measure the velocity correlation of concentrated bacterial suspensions at different H without shear. Our experiments demonstrated the linear relation between the velocity correlation length and the size of the system, a relation referred in our current study.

In Ref. 41 (the original Ref. 44) titled "Imaging the emergence of bacterial turbulence: Phase diagram and transition kinetics", we addressed the specific question on how random bacterial motions at low concentrations transition into collective swimming, i.e. active turbulence, with increasing bacterial concentrations. Particularly, we quantitatively measured the critical transition concentration as a function of bacterial swimming speeds and the fraction of active swimmers in mixtures of mobile and immobile bacteria. We then compared our results with the prediction of the kinetic theories on the critical transition concentration, which showed a good agreement. In addition, we have also explored two different kinetic pathways of bacterial turbulent transition by

triggering the activity of bacteria with light. We showed that the two kinetic pathways form good analogy to the process of nucleation and growth and spinodal decomposition in equilibrium phase transitions. We adapted a similar approach to trigger the bacterial turbulence in the last section of the current study in order to explore the temporal development of density fluctuations during the transition towards active turbulence.

We should emphasize that, in both these two previous studies, we only measured the velocity fields and the velocity correlations of bacterial suspensions using PIV, which are similar to all the other previous experimental works from other researchers (see e.g. Ref. 7, 14, 15, 17, 18, 33, 42, 49–53). In contrast, we focused on *density fluctuations* of bulk bacterial suspensions in the current work using a completely different experimental approach based on the Beer-Lambert law. To the best of our knowledge, density fluctuations have not been experimentally studied previously in not only 3D bacterial suspensions but also 3D active fluids in general. Our results uncovered the physical properties of the density fluctuations of bulk bacterial suspensions including their scaling relation and length-scale dependence and revealed the unexpected density fluctuations–energy spectra coupling. The results reported in this manuscript represented the most important findings of our group out of our intensive research efforts on bacterial suspensions over the last 7 years. Indeed, the figures from our current manuscript, which we have posted on arXiv while submitting this manuscript, have already been cited and discussed by the recent review summarizing the key progress in the study of active turbulence over the last two decades (see Fig. 1d in "Active Turbulence" by Alert et al., arXiv:2104.02122 and the associated discussions).

While we fully agree with the reviewer that we need to be clear on the previous findings of our own works, we feel that introducing these works in details in Introduction would distract the focus of the introduction, as our previous works are not directly related to the main theme of the current study on density fluctuations. Rather, we should use the concise space of Introduction to reference works from other research groups that directly addressed the issue of density fluctuations in active fluids. Hence, instead of presenting a lengthy discussion of our own works in Introduction, we explained our previous results and their relation to the current work in the revised main text of the manuscript when and where they were cited.

Specifically, we have added the following discussion for Ref. 37 in pg. 5:

"Our previous study on high-ϕ bacterial suspensions in a planar Couette cell with an adjustable height have shown that the velocity correlation length is linearly proportional to the size of the system (*37*), which is set by the minimum dimension of the sealed chamber H = 140 μm in our current study."

In addition, we have added the new comment on Ref. 37 in pg. 8:

"This finding, in combination with our previous result on the linear relation between the velocity correlation length and the system size (*37*), challenges the popular view of dense bacterial suspensions as active polar fluids (*4*, *33*, *35*, *50*)."

For Ref. 41, we have added the following discussion in pg. 5:

"…, where ϕ_c is the transition concentration to active turbulence. ϕ_c = 4.05% in our experiments, quantitatively agreeing with our previous experiments as well as the prediction of kinetic theories (*41*)."

and the following discussion in pg. 11:

"Taking the advantage of the light-powered bacteria, we trigger the onset of bacterial turbulence by suddenly turning on the light illumination on high-ϕ bacterial suspensions at t = 0, similar to the approach we used previously in (*41*)."

3. The authors sort of imply that anomalous fluctuations occur at concentrations below the critical value for the instability that leads to collective motion. However, it seems these fluctuations are not anomalous in the sense defined above. The fluctuations are supposed to follow a power law but only over a very limited range of lengths from 0.3 of the bacteria length to about twice the bacterium length. I do not understand how it can be considered relevant to look at density fluctuations at 1/3 the bacterium size. This is not a large enough region to define a number density and one could not expect a scaling representative of long range fluctuations on this scale. Any spectrum that varies one might plot on a log-log plot over a very limited range and get a power law but the significance of that is doubtful. In fact looking at fig 3 and focusing on phi=2.4%, I do not see any region that does not have significant downward curvature unless such points are being hidden under the other colors at very small lengths.

Thanks for pointing out this confusing point. We feel that there may be a misunderstanding of the definition of the giant number flucatuions due to the confusing writing of our original manuscript. To address the question of the reviewer, we like to clarify three points:

First, the anomalous density fluctuations, i.e. the giant number flucatuions referred in our study, do not necessarily mean a power-law relation between ΔN and $\sqrt{N}$. Any fluctuations with standard deviation ΔN growing faster than $C\sqrt{N}$ are considered as giant number fluctuations (see the definition in the first paragraph of our revised Introduction), where C is a constant independent of N. For example, one could have ΔN grows exponentially with $\sqrt{N}$, which should be considered as giant number fluctuations by definition. To highlight the existence of giant number flucatuions in bacterial suspensions, we plot $\Delta N/\sqrt{N}$ versus N (or equivalently $\Delta N/l$ versus l^2 since $l \sim \sqrt{N}$) in Fig. 3. If ΔN grows linearly with $\sqrt{N}$ *without* giant number fluctuations, $\Delta N/\sqrt{N}$ would be a constant independent of N (or l) and the data should be a horizontal constant line. However, our samples at all concentrations show a clear increase of $\Delta N/\sqrt{N}$ with l, when l is less than the density correlation length. Hence, the results demonstrate the existence of giant number flucatuions at small scales, independent of whether we perform the power-law fitting or not. Particularly, we hope it is sufficiently clear that the data at ϕ = 2.4% questioned by the reviewer is not a constant line, which shows an unambiguous increasing trend at small l. Thus, by definition, there exist giant number fluctuations in low-ϕ suspensions at small scales. More interestingly, all the data at different ϕ seem to converge to a master curve at small l (which is why points at small l are hidden

under the other colors as pointed out by the reviewer), indicating a common trend of density fluctuations at small l for suspensions of all different ϕ. Again, the observation is independent of any power-law fitting.

Second, motivated by theoretical predictions (e.g., Ref. 4 and 24), power-law scaling has been frequently used in the existing experimental literature to quantify the degree of giant number fluctuations (e.g. Ref. 10, 12, 13, 14, 16–20). However, as correctly pointed out by the reviewer, it is always questionable if the power-law relation truly holds experimentally, where the dynamic range of length scales that can be explored experimentally (or numerically) is unavoidably limited. Here, we followed this convention and fit our data with a power-law relation, which would allow us to quantify the increasing trend of $\Delta N/\sqrt{N}$ and compare our results with existing theories and 2D experiments in the literature. Nevertheless, we like to emphasize again that the conclusion of our study on the existence of the scale-dependent giant number flucatuions in suspensions of different ϕ by no means relies on the power-law fitting. To address the concern raised by the reviewer, we have explicitly discussed the issue associated with the limitation of the power-law fitting in the first paragraph of pg. 7 of the revised manuscript:

"Note that although the accuracy of the power-law fitting is limited due to the finite length scale of our experiments especially for low-ϕ suspensions, the existence of the scale-dependent GNF in bacterial suspensions of different concentrations is robust and does not rely on the fitting."

Third, the volume of the subsystem under consideration is $V = l^2 d$, where l is the side length of the subsystem within the imaging plane and d is the depth of field normal to the imaging plane. We estimate d to be about 6 μm based on the optical parameters of our microscope system (see SM Sec. 3.1.1). At the smallest length of 1/3 of bacterial length $l = l_b/3 = 1$ μm chosen in our analysis, where $l_b \approx 3$ μm is the average length of bacterial body, $V = 6$ μm^3, which is still sufficiently large to hold up to six bacteria in the subsystem. Note that the volume of bacteria is $V_b = \pi(w_b/2)^2 l_b \approx 1$ μm^3, where $w_b = 0.65$ μm is the average width of bacterial body. Hence, even at the smallest length scale of our analysis, local correlation between multiple bacteria can still exist within a subsystem, which was indeed detected in our experiments at small l. To clarify the confusion, we have added the above explanation at the end of SM Sec. 3.1.1.

4. Having some scaling of the density on scales smaller than the instability length scale which seems to be on the order of the channel depth would not be surprising. We would not expect random number density distribution in an unstable system. So it seems that neither the case below the critical phi nor above the critical phi is really "anomalous" in the sense of long-range correlations in a stable system. The behavior above the instability could however be a feature that one could look for in simulations.

As commented above, the kinetic theories of active suspensions have predicted the existence of a long-wavelength hydrodynamic instability of the isotropic state of bacterial suspension above the critical concentration ϕ_c (Ref. 45–48), which develops into the full active turbulence beyond the

linear regime based on the numerical solution of the kinetic equations (Ref. 46). Interestingly, the linear stability analyses performed in Ref. 45–48 all showed that, even after a suspension has become unstable and developed a long-range orientational correlation, the density of the suspension still remains *uniform* in the linear regime. The strong density fluctuations are the consequence of the nonlinear dynamics of the kinetic equations beyond the linear regime. Hence, an unstable system with the long-range correlation does not necessarily gives rise to density fluctuations within the framework of kinetic theories. In the linear regime, density remains uniform with the long-ranged orientational order of bacteria. Hence, revealing giant density fluctuations in concentrated bacterial suspensions in experiments provided a nontrivial verification of the hypothesis of the kinetic theories.

It is certainly interesting to run large-scale simulations to explore the collective dynamics of dense bacterial suspensions, which would allow us to assess quantities that cannot be easily obtained in experiments. Such simulations would complement, rather than replacing, the experimental study reported here. The concentrated bacterial suspensions used in our experiments contain more than a billion bacteria, which is hard, if not impossible, to simulate with the full hydrodynamics given the high computational cost of simulations. In addition, to the best of our knowledge, none of the existing numerical schemes can fully capture the complex near-field bacterial flow and the steric interaction between bacteria even on the level of two interacting bacteria. Thus, experiments would allow us to examine the dynamics of concentrated bacterial suspensions in the bulk limit and reveal the effect of the complicated inter-bacterial interactions on the emergent collective behaviors of bacterial suspensions.

5. The volume fraction fluctuation measurement is based on light attenuation. Since the cells are nonspherical and the instability involves changes of orientation, how do we know that what is being observed is really density fluctuations rather than orientation fluctuations? Is there a test that can be made for this?

This is an important and interesting point, which we had not thought about previously. It is certainly hard to design a control experiment, where one can eliminate the density flucatuions caused by the active turbulent flow, but at the same time maintain the fast rotation of bacteria induced by the turbulent flow. Hence, instead of running a direct control, we propose here two independent experimental and theoretical evidence, supporting the hypothesis that light intensity variations arise from density flucatuions, instead of the fluctuations of bacterial orientation.

First, the anti-correlation between the velocity correlation time and the density correlation time provides indirect evidence on the origin of intensity variations. As nicely illustrated by the scaling argument provided by the reviewer below in Question 9, the temporal change of intensity is inversely related to the magnitude of local bacterial velocity (Eq. (R4) below). While it is questionable whether there is a direct link between the translational and rotational motions of bacteria, it is straightforward to think that local bacterial velocities control the movement of bacterial clusters of different densities, therefore resulting in the variation of local light intensity.

Second, we have also done a simple calculation to show that the change of the orientation of bacteria does not cause the variation of local light intensity. Let's consider two extreme orientations of a bacterium with respect to the imaging plane: (*i*) the bacterium normal to the imaging plane of total area A (Fig. R1A); and (*ii*) the bacterium parallel to the imaging plane (Fig. R1B). Since the depth of field is larger than the length of bacterial body, we approximate the light to be nearly collimated around the bacterium. For simplicity, we also approximate the bacterial body as a cylinder, where the length of the cylinder l_b equals to the length of bacterial body and the diameter of the cylinder w_b equals to the width of bacterial body.

In Case (*i*), the light of intensity I_0 passes through the length of bacterial body (Fig. R1A). The attenuation of the light underneath the bacterium follows the Beer-Lambert law:

$$\frac{I_\perp}{I_0} = e^{-\mu l_b} \approx 1 - \mu l_b,$$

where $I_\perp$ is the light intensity underneath the bacterium and μ is the absorbance of bacteria per unit length. The approximation in the last step is taken due to the weak absorbance and the small length l_b as verified by experiments (Fig. 1D). The cross-section of the bacterium in the imaging plane is $S_\perp = \pi w_b^2/4$. Thus, the average light intensity over the image plane is

$$\langle I \rangle_\perp = \frac{I_\perp S_\perp + I_0(A - S_\perp)}{A} = I_0\left(1 - \frac{\pi \mu l_b w_b^2}{4A}\right). \quad \text{(R2)}$$

In Case (*ii*), the light of intensity I_0 passes through the width of bacterial body, whose thickness varies with the position along the cross-section of the bacterium (Fig. R1C). The light path at position x is $l(x) = 2\sqrt{w_b x - x^2}$. The Beer-Lambert law dictates the light intensity at position x, $I_\parallel(x)$:

$$\frac{I_\parallel(x)}{I_0} = e^{-\mu l(x)} \approx 1 - 2\mu\sqrt{w_b x - x^2}$$

The maximum cross-section area of the bacterium parallel to the imaging plane is $S_\parallel = l_b w_b$. The average light intensity can then be calculated as

$$\langle I \rangle_\parallel = \frac{\int_0^{w_b} I_\parallel(x) l_b dx + I_0(A - S_\parallel)}{A} = I_0\left(1 - \frac{\pi \mu l_b w_b^2}{4A}\right). \quad \text{(R3)}$$

Note that we have used the relation $\int_0^{w_b} 2\sqrt{w_b x - x^2}dx = \pi w_b^2/4$.

Comparing (R2) and (R3), we have $\langle I \rangle_\perp = \langle I \rangle_\parallel$. Indeed, the above calculation can be easily extended to an object of arbitrary shape, which gives

$$\langle I \rangle = I_0\left(1 - \frac{\mu}{A} V\right),$$

where $\langle I \rangle$ is the average light intensity over the imaging plane and V is the volume of the object. Importantly, $\langle I \rangle$ only depends on the volume of the object, independent its orientation with respect to the direction of the light beam. Hence, bacterial orientation does not affect the average local light intensity measured in experiments.

To address the question in the revised manuscript, we have added the derivation in SM Sec. 3.1.4 of the revised supplementary materials and added the following comment in the experimental section of the main text in pg. 4:

"Furthermore, a simple calculation on the light attenuation of bacteria of different orientations can show that the change of bacterial orientation does not cause the variation of local average light intensity measured in experiments (SM 3.1.4)."

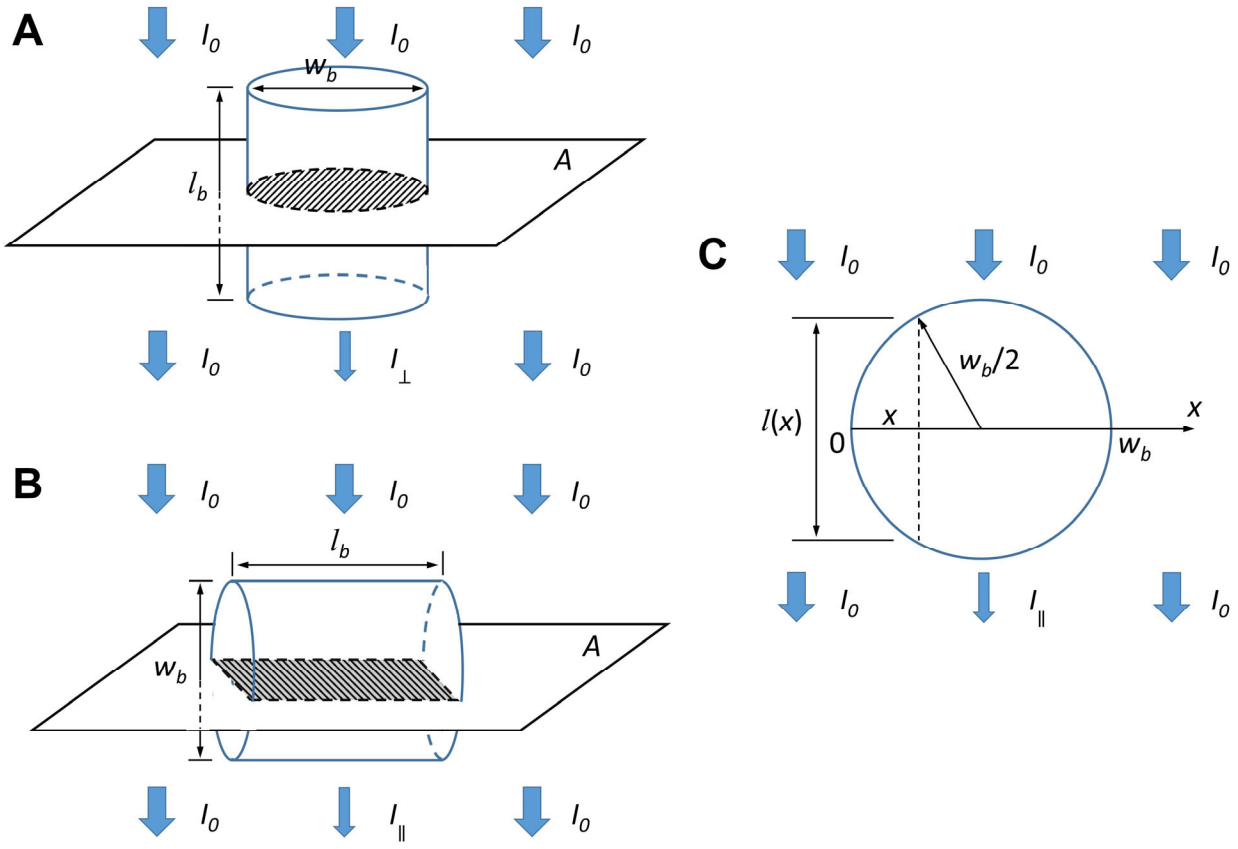

Fig. R1 Schematics showing the orientations of a bacterium with respect to the imaging plane. (A) A bacterium normal to the imaging plane. (B) A bacterium parallel to the imaging plane. (C) A side view of the cross-section of the bacterium in (B). The light path $l(x)$ varies with x, where the x-axis is within the imaging plane and normal to the long axis of the bacterial body.

6. In figure 2, the authors should remove the reference to "theory" which might lead one reading the caption alone to think that there is some basis for the "theory". The discussion in the text appropriately makes it clear the theory has no basis of applicability here. It is for a different type of system and would only apply if that system were stable which it is not.

We believe the reviewer actually referred to Fig. 3, instead of Fig. 2, where we have compared experimental results with theory in the original manuscript.

Following the suggestion of the reviewer, we have remove the reference to the theory in the caption of Fig. 3.

7. The authors refer to a two-point density spatial autocorrelation as a two-point density spatial correlation and refer to a two-time density temporal autocorrelation as a density autocorrelation. This might suggest to a reader that the former is not an autocorrelation when it is.

Thanks for pointing out the confusing point. We have changed the names of the correlations in the revised text following the suggestion. To avoid the confusion, we have also provided the exact mathematical definition of the density/velocity spatial autocorrelation and the density/velocity temporal autocorrelation in the SM Sec. 2.1 of the revised manuscript, which are given below:

Velocity spatial autocorrelation function,

$$C_v(\boldsymbol{r}) = \frac{\langle \boldsymbol{v}(\boldsymbol{r_0}, t_0) \cdot \boldsymbol{v}(\boldsymbol{r_0} + \boldsymbol{r}, t_0) \rangle_{\boldsymbol{r_0}, t_0}}{\langle \boldsymbol{v}(\boldsymbol{r_0}, t_0) \cdot \boldsymbol{v}(\boldsymbol{r_0}, t_0) \rangle_{\boldsymbol{r_0}, t_0}}$$

Velocity temporal autocorrelation function,

$$C_v(t) = \frac{\langle \boldsymbol{v}(\boldsymbol{r_0}, t_0) \cdot \boldsymbol{v}(\boldsymbol{r_0}, t_0 + t) \rangle_{\boldsymbol{r_0}, t_0}}{\langle \boldsymbol{v}(\boldsymbol{r_0}, t_0) \cdot \boldsymbol{v}(\boldsymbol{r_0}, t_0) \rangle_{\boldsymbol{r_0}, t_0}}$$

Density spatial autocorrelation function,

$$C_n(\boldsymbol{r}) = \frac{\langle n(\boldsymbol{r_0}, t_0) n(\boldsymbol{r_0} + \boldsymbol{r}, t_0) \rangle_{\boldsymbol{r_0}, t_0}}{\langle n(\boldsymbol{r_0}, t_0) n(\boldsymbol{r_0}, t_0) \rangle_{\boldsymbol{r_0}, t_0}}$$

Density temporal autocorrelation function,

$$C_n(t) = \frac{\langle n(\boldsymbol{r_0}, t_0) n(\boldsymbol{r_0}, t_0 + t) \rangle_{\boldsymbol{r_0}, t_0}}{\langle n(\boldsymbol{r_0}, t_0) n(\boldsymbol{r_0}, t_0) \rangle_{\boldsymbol{r_0}, t_0}}$$

where $\langle \dots \rangle_{\boldsymbol{r_0}, t_0}$ denotes an average over all possible positions and times. Since the flow is isotropic, we consider only the spatial correlation as a function of the magnitude of the position vector $r = |\boldsymbol{r}|$ by further averaging $C_v(\boldsymbol{r})$ and $C_n(\boldsymbol{r})$ over a constant r. The number density, n, is proportional to the local light intensity, which is shifted so that $\langle n \rangle_{\boldsymbol{r_0}, t_0} = 0$.

8. When showing correlations of phi and of velocity I think it is incomplete to not also show how the variance of the velocity and the variance of the volume fraction (measured at some length scale slightly larger than the bacteria size so one can define such a quantity) depends on the mean phi. This would give one a practical understanding of how large the "giant" density fluctuations really are. Perhaps one could infer this info from delta(N) at a small l but it would

be tedious to do the calculation. In fact, one could give delta(phi) vs l rather than delta(N) vs l and still see a power law.

Following the suggestion, we have plotted the standard deviation of the local volume fraction $\Delta\phi = \Delta N V_b/V$ versus l^2 for bacterial suspensions of different concentrations (Fig. R2), where $V_b \approx 1\ \mu m^3$ is the volume of bacteria and $V = l^2 d$ is the volume of the subsystem. l is the side length of the subsystem and d is the depth of field. Note that for isotropic equilibrium systems following the central limit theorem, $\Delta N \sim \sqrt{N}$. Thus, $\Delta\phi$ should scale as $\sim 1/l$ at given ϕ. Any fluctuations with $\Delta\phi$ decreasing slowly than $1/l$ would be considered as giant density fluctuations by definition. As shown in Fig. R2, suspensions at different ϕ all show the slow decay at small l, indicating the scale-dependent giant number fluctuations.

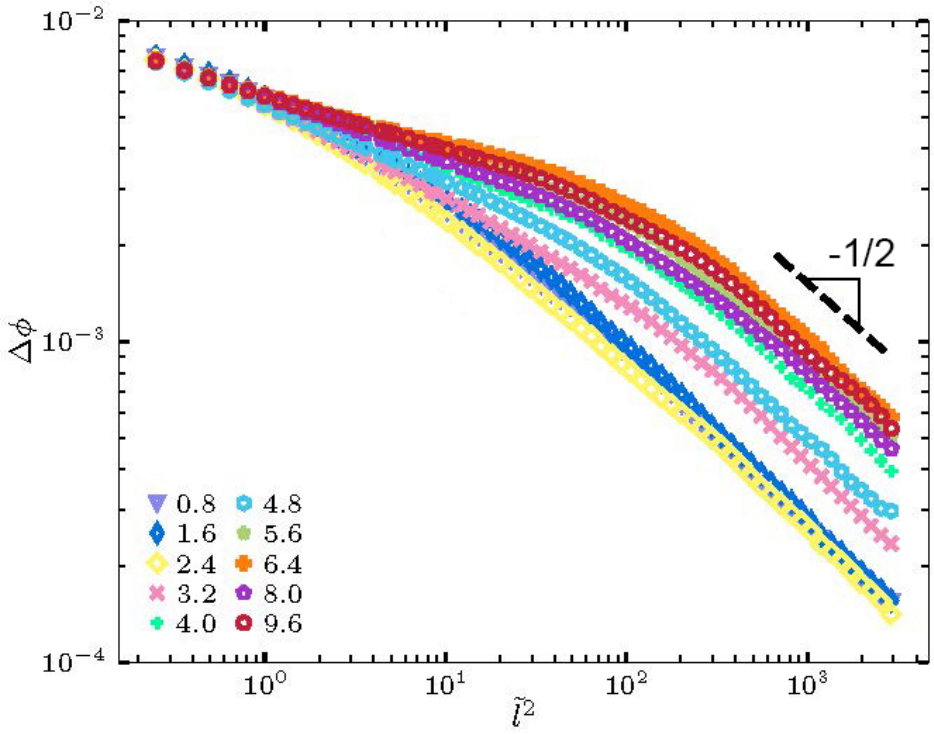

Fig. R2 The standard deviation of bacterial volume fraction $\Delta\phi$ in a subsystem of side length l as a function of the area of the subsystem l^2 for suspensions of different volume fractions ϕ. The dashed line has a slope of -1/2, which indicates the fluctuations controlled by the central limit theorem with $\Delta\phi \sim 1/l$.

9. The explanation for the reduction of the density correlation time with increasing phi could be more complete. The authors mention there is a reduction due to an increasing velocity variance (without actually showing the increase). However, one could think of tau_n=lambda^2/D where lambda is the length scale which Is either the pixel size of the length scale of the fluctuation and D=<v'^2>tau_v is the hydrodynamic diffusion of bacteria. The increase in the correlation time of the velocity then helps to decrease the correlation time of number density. In a very dilute

suspension this D would revert to the run and tumble diffusivity and the authors could compare to that estimate.

We thank the reviewer for the insightful comment, which indeed provides a good estimate of the density correlation time of bacterial suspensions in the dilute and concentrated limit.

Specifically, following the suggestion of the reviewer, we write the density correlation time τ_n as

$$\tau_n = \frac{\lambda_n^2}{D} = \frac{\lambda_n^2}{\langle v \rangle^2 \tau_v}, \tag{R4}$$

where λ_n is the density correlation length, characterizing the length scale of density inhomogeneity in the system. Velocity fluctuations are treated as a stochastic process with an effective diffusion coefficient $D = \langle v \rangle^2 \tau_v$, where $\langle v \rangle$ is the average local velocity and τ_v is the velocity correlation time.

For high-concentration bacterial suspensions with active turbulence, λ_n is naturally set by the size of bacterial clusters at the scale of turbulent vortices. Since bacteria are well aligned in the clusters, the average local velocity should equal to the velocity of the swirling vortices. Thus, $\lambda_n / \langle v \rangle \sim \tau_v$, which leads to $\tau_n \sim \tau_v$ as observed in experiments (Fig. 2F).

For low-concentration bacterial suspensions, λ_n is set by the size of individual bacteria l_b (Fig. 2E), whereas τ_v is given by the characteristic time scale of bacterial swimming, $\tau_v = \tau_b \equiv l_b / v_0$ (Fig. 2F). It is hard to estimate the average local velocity for low-concentration suspensions, as there is no general alignment of bacterial orientation. Assuming an isotropic distribution of bacterial orientation at low concentrations, $\langle v \rangle$ would be zero if all bacteria have the same swimming speed v_0. Thus, the average velocity must arise from the fluctuations of the swimming speed of individual bacteria around the mean, i.e., $\langle v \rangle \sim \Delta v = \sqrt{\langle v^2 \rangle - v_0^2}$, where Δv is the standard deviation of bacterial swimming speed. Given $\tau_n \approx 10\tau_b$ from experiments (Fig. 2F), we have $\langle v \rangle = \Delta v \approx 0.32 v_0 = 4.7$ μm/s based on Eq. (R4), agreeing well with experimental measurements $\Delta v = 3$ μm/s. Thus, the variations of the swimming speed of individual bacteria lead to a small but finite average local bacterial velocity, which gives rise to the long density correlation time at small ϕ.

To avoid more technical discussions in the main text as criticized by Reviewer 1 (see Question 1 from Reviewer 1), we added the above scaling argument in the revised supplementary materials (SM Sec 2.2). In the revised main text, we added the following sentence at the end of the second paragraph of pg. 5:

"A simple scaling argument can be formulated based on the above picture, which provides a good estimate of the density correlation time in the low-ϕ and high-ϕ limit (SM Sec. 2.2)."

10. The authors refer to "paradigmatic example of 3D wet active fluids" several times without really articulating the justification for this claim.

The classification of active fluids into dry and wet types was presented in the influential review by Marchetti *et al.* (Ref. 4). Bulk bacterial suspensions are listed as one of a few examples of wet active fluids (Table 1 of the paper). Furthermore, the starting sentence of the abstract of the review by Saintillan and Shelley (Ref. 8) also states that "Active suspensions, of which a bath of swimming microorganisms is a paradigmatic example, denote large collections of individual particles or macromolecules capable of converting fuel into mechanical work and microstructural stresses". Indeed, bacterial suspensions are consistently referred to as one of the most important experimental realizations of wet active fluids (e.g. the most recent review by Alert et al., arXiv:2104.02122).

To avoid the redundancy, we removed all the mentioning of bacterial suspensions as a paradigmatic example of wet active fluids, saving only the first one in the Introduction in pg. 3 of the revised manuscript. Moreover, we have cited Ref. 4 and 8 to support this statement immediately after this first and only mentioning in the revised text. We kept the statement in the abstract of the manuscript, where we cannot add references.

11. The density-flow coupling section on p 8 is awkwardly written. Normally in an experimental paper one endevours to show the experimental evidence and how it is obtained and only afterward explain its significance relative to theoretical ideas. Here, the section starts with a theory and the theory is explained as if we expect it to be true and then the experiment is show to show it is not true. And then another theory is offered up. This needs to be written more smoothly. There are a number of possible problems with (2) and the correlation suggested based on it. Eqn (2) assumes a single bacterium velocity at a point in space so it leaves out run and tumble diffusion or other velocity fluctuations on the intercell scale. The Saintillan simulations omitted tumbling. They used slender rod models that might have had less actual cell collision than the real system. The idea that n should be correlated with div(v n d) at the same time also assumes that one has a standing wave rather than a traveling wave.

We agree with the reviewer that the organization of the original writing was not ideal and indeed confusing. Following the suggestion, we have reversed the order of the writing in the revised manuscript, where we discussed our experimental findings first before comparing our results with theoretical predictions.

Note that our theory is based on a modified version of the mass conservation equation proposed by Saintillan and Shelley (Eq. 11 in Ref. 46). Particularly, as pointed out by the reviewer, the original equation suggested by Saintillan and Shelley assumed a single bacterial velocity at a point in space, which is a constant denoted as U_0 in Eq. 11 of Ref. 46. Since U_0 is a constant, it is taken outside the divergence on the right of the conservation equation. In contrast, we take a mean-field

approach and treat the velocity as the local mean bacterial velocity denoted as $\bar{v}_0$ in our equation, which is not only a function of position $\boldsymbol{x}$ and time t, but also a function of local density n. The averaging process itself takes into consideration the effect of bacterial tumbling and velocity fluctuations. We do not include the diffusive term $D\nabla^2 n$ in the conservation equation, as such a term is always small compared with advection, especially in the turbulent regime that we are interested in.

We have modified our discussion of the mass conservation equation in the revised manuscript to highlight the average nature assumed in our study.

"$\bar{v}_0(\boldsymbol{x},t)$ is the local average bacterial swimming speed, which may depend on the local density n."

Lastly, we have also commented in the revised writing that the simulation by Saintillan and Shelley assumed the standing-wave solution of 2D active turbulence.

"Saintillan and Shelley showed numerically that the local density n anti-correlates with the divergence of the relative bacterial flux $\nabla \cdot (n\bar{v}_0\boldsymbol{d})$ for the standing-wave solution of 2D active turbulence (*46*)."

12. The authors show in fig 5C instead what I take to be a positive correlation between phi'=phi-<phi> and KE'=KE-<KE> where KE=v^2 so I take it that C1=<phi'KE'> although it is not explicitly defined. It should be. I think that this correlation is interesting and is not identical to the later result about similarities between <v'(x1)v'(x2)> or its Fourier transform and <phi'(x1)phi'(x2)>. The latter are saying that the autocorrelations of v and phi are similar to each other, while C1 is a cross-correlation. In a system of passive particles such as inertial particles in turbulence, a rapid (inertial) granular flow, or a slow granular flow with pressure and enduring contacts, the volume fraction would be negatively correlated with the KE. Regions that flow and have energy and disorder expand. Intuitively I would expect an active fluid would have dense regions of aligned swimmers enabling swimmers to swim together without many collisions and thus have a high KE so a positive correlation is obtained. For what systems has this been demonstrated in theory or simulation (only dry 2D?). If so someone really should seek it in wet 3D studies. I wonder if one needs denser systems. The squirmers of Isikawa? Or are there studies of active colloids that might show it.

C_1 in Fig. 5C is defined as

$$C_1 = \frac{\langle(\delta N - \overline{\delta N})(E - \bar{E})\rangle}{\sigma_{\delta N}\sigma_E},$$

where δN is the temporal *fluctuations* of local density and E is the local kinetic energy. $\bar{A}$ indicates the spatial average of A at a fixed time, whereas σ_A indicates the standard deviation of A. $\langle A\rangle$ denotes the average of A over all the positions and times. The definition has been given as Eq. (15)

of the supplementary materials (SM) and explained in great details in SM Sec. 3.4.1. We left the mathematical definition of C_l in SM, rather than including it in the main text, to avoid overly technical discussion as criticized by the first reviewer (see Question 1 of Reviewer 1). It should be emphasized that C_l measures the cross-correlation between the *fluctuation* of local density δN (or equivalently $\delta\phi$ as the length scale is fixed in this analysis) and the local kinetic energy $E = v^2/2$, instead of the cross-correlation between the local density N (or ϕ) and E as commented by the reviewer.

To avoid the confusion and make sure the interested readers know where to see the definition, we have explicitly state in the revised manuscript:

"Finally, to illustrate the correlation between density fluctuations and kinetic energy at a given scale in real space, we calculate the correlation of local density fluctuations and kinetic energy at the smallest length scale of our PIV analysis, i.e., the step size of PIV at $l = 2.75l_b$ (see SM Sec. 3.4.1 for the definition)."

The comparison between passive particles in turbulence, granular flows and active fluids suggested by the reviewer is indeed very interesting. For 2D systems of spherical active particles such as active colloids, the velocity of active particles decreases with increasing particle density similar to the case of granular flows, which leads to the well-studied phenomenon of motility-induced phase separation with a coexistence of a dense cluster phase and a dilute gas phase (see the review Ref. 21 by Cates and Tailleur). Very recent theoretical and numerical work by Worlitzer *et al.* (New J. Phys. **23**, 033012 (2021)) extended the continuum model of Dunkel *et al.* on active polar fluids (Ref. 33) by including the density-velocity coupling. Nevertheless, they again only considered the case where the velocity decrease with increasing particle density. Their numerical simulations showed a rich variety of emergent phases, including the motility-induced phase separation with domains of dynamically changing irregularly shaped boundaries.

For experimental 2D systems, early work by Zhang *et al.* investigated the collective motion of a monolayer of bacteria sliding on agar surfaces (Ref. 14). They showed that the average speed of isolated bacteria is slow about 15 μm/s and their speed increases with increasing bacterial cluster size, saturating at 40 μm/s for bacteria in clusters of more than 20 bacteria. The observation can be attributed to the enhanced bacterial alignment in clusters, as suggested by the reviewer. To the best of our knowledge, the density-velocity coupling has not been examined in 3D wet active fluids. While the steric interaction between particles controls the dynamics of bacterial motions in 2D, the long-range hydrodynamic interaction dominates the steric interaction in 3D systems, which may lead to qualitatively different behaviors. To address the question of the reviewer, we have analyzed the direct cross-correlation between local bacterial density and kinetic energy in bulk bacterial suspensions in our experiments, which is defined as

$$C_3 = \frac{\langle (N-\bar{N})(E-\bar{E}) \rangle}{\sigma_N \sigma_E}.$$

Note that the local bacterial number N is proportional to the local density n (or equivalently the local average light intensity) as the size of the local subsystem is fixed.

Figure R3 below shows the results. Local density and kinetic energy show small but positive correlations when $\phi < \phi_c$. A high bacterial density enhances local bacterial alignment and therefore increases local flow velocity, a feature similar to that observed in 2D bacterial swarming on agar substrates discussion above. However, when $\phi > \phi_c$ in active turbulence, local density and kinetic energy become anti-correlated. The finding is reminiscent of the preferential accumulation of passive inertial particles in high-*Re* turbulence as pointed out by the reviewer. Heavy passive inertial particles concentrate at convergence zones in high-*Re* turbulence, where multiple vortices interact to form a saddle-like flow with low flow velocity. Nevertheless, since the particle Stokes number of bacteria is $St \sim 10^{-5}$, the inertial effect that drives particle preferential accumulation in high-*Re* turbulence cannot be present in bacterial suspensions. Instead, we hypothesize that the anticorrelation arises from the unique features of bacterial locomotion in shear flow such as rheotaxis and shear-induced trapping of bacteria (Ref. 62, 63). More works are certainly needed to reveal the origin of the intriguing density-flow correlation, which are beyond the scope of the current study focusing on density fluctuations and will be the subject of our future investigations.

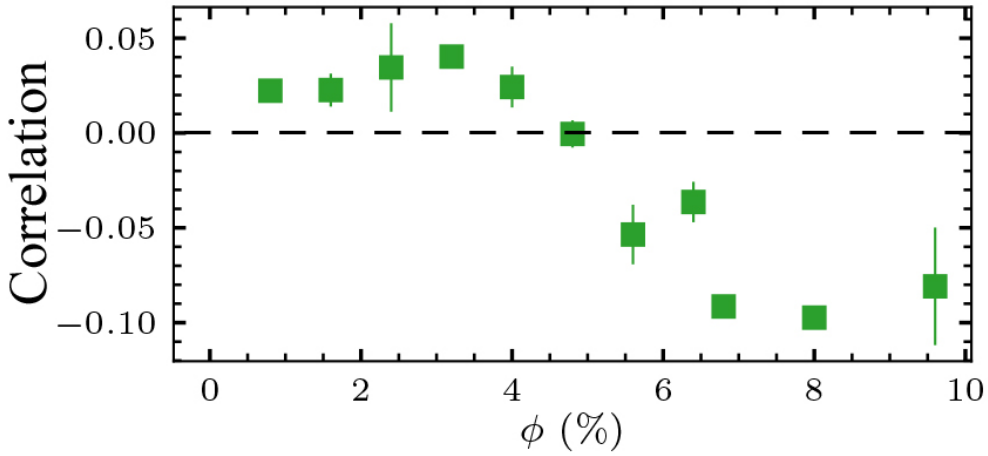

Fig. R3 Correlation between local bacterial density and kinetic energy for suspensions of different volume fractions. The dashed line indicates zero correlation. The transition concentration to active turbulence is $\phi_c = 4.05\%$.

We included the new correlation result as a new paragraph in the revised manuscript (pg. 11, first paragraph). The definition of the density-flow cross-correlation is further given in SM 3.4.3 of the revised supplementary materials. As pointed out by the reviewer, the correlation between local density (not local density *fluctuations*) and local kinetic energy is very different in nature compared with our analysis on the coupling between density fluctuations and energy spectra. Because the focus of our manuscript is on density fluctuations and the space of our manuscript is limited, we will leave more analyses on the density-flow coupling to our future study.

13. The discussions of how velocity fluctuations lead to density fluctuations in p 9 and 10 seemed somewhat incomplete in that it seemed like "one-way coupling", the velocity creating the density fluctuation and not vice versa. It also seemed like it might be reinventing some ideas that would have been developed for passive scalar spectra in turbulence.

The reviewer is correct that we consider a one-way coupling, where the velocity field induces the density fluctuations and not vice versa. We use this simple approximation to explain the initial growth of density fluctuations from the state of uniform density in the linear regime.

To be clear on the approximation taken in our analysis, we have added the following sentence at the end of the first paragraph of pg. 10 of the revised manuscript:

"The simple argument above assumes a one-way coupling, where the turbulent flow triggers density fluctuations and not vice versa. This simple assumption is valid at early time during the initial growth of density fluctuations from the state of uniform density in the linear regime. As density fluctuations build up, the back influence of density inhomogeneities on the turbulent flow may not be ignored."

As mentioned in our answer to the previous question, the particle Stokes number of bacteria is $St \sim 10^{-5} << 1$. Thus, if we treat bacteria as passive tracers, they should simply follow all the motions of the turbulent flow and disperse as fluid elements. Since the fluid is incompressible, the turbulent flow would not create any density fluctuations of bacteria under this assumption. Thus, the density fluctuations observed in our experiments must be the consequence of the self-propelled motion of bacteria, lacking in the transport of non-inertial particles in high-*Re* turbulence. Note that in our discussion of the rise of density fluctuations in pg. 9 and 10, the density fluctuations are driven by $\nabla \cdot (n\bar{v}_0 \boldsymbol{d})$, where $\bar{v}_0$ is average bacterial velocity, instead of fluid velocity. Moreover, as argued above in the previous questions, the density-flow coupling shown in Fig. R3 likely arises from rheotaxis and shear-induced trapping of bacteria. Both effects are unique to bacterial locomotion in shear flow, which do not exist for passive tracers in high-*Re* turbulence.

With that being said, we do agree with the reviewer that the coupling between energy spectra and density flucatuions in active turbulence may be analogous to some processes in high-*Re* turbulence. Although we have come up with the idea independently, there may be similar approaches/ideas in the study of high-*Re* turbulence. Nevertheless, when we searched the literature on passive scalar spectra in turbulence and related studies, we could not find works discussing explicitly the link between density fluctuations and energy spectra. As our technical expertise is not in the field of high-*Re* turbulence, considering the vast body of turbulence literature, it is certainly possible that we miss important papers. We will appreciate if the reviewer could generously provide us some relevant references, which would be very helpful not only for completing the current manuscript, but also for our future studies.

Lastly, in the attempt to address the concern of the first reviewer who commented that our original manuscript is too technical for *Sci. Adv.*, we have removed the tentative scaling argument for the density fluctuations–energy spectra coupling in the revised manuscript, which we feel is speculative and certainly technical in nature.