Response to Reviewer 1:

We thank Reviewer 1 for his/her very positive and insightful remarks and detailed suggestions. They enabled us to improve our manuscript. Remarks of Reviewer 1 are denoted in *italics*.

"In this study, the authors attempt to develop a unique coarse-grained description of signaling dynamics involving cells that produce and respond to a diffusible signal - which they previously termed as "sense-and-secrete" cells (from Youk and Lim 2014).

Their framework consists of formalized description of two major components involved in cell-cell communication systems. The first is how a cell responds to a certain concentration of a signal - the signal either activates or suppresses its own production, depending on whether it crosses a threshold. The second component is the relative contribution of the producing cell and its neighbors to the signal concentration perceived by the cell. Whether a cell remains primarily autonomous or neighbor-driven depends on the local density of cells (number of neighbors) and the signaling length scale. They quantify the combination of these two factors using a lumped function, "signaling strength". The signaling strength increases with both the local density and the signaling length scale. They further show that this theoretic formulation allows the prediction on whether a more complex system would generate interesting collective dynamics.

General comments
I find the conceptual framework of the work refreshing. If well established, it can provide quantitative description of essential features of dynamics involving cell-cell communication. This framework can be advantageous in providing quantitative insights without invoking more detailed, molecular-level based description. For instance, a full-fledged model of such a process could entail an agent-based model that accounts for the details of signal production, degradation, and diffusion, as well as response to the signal. The analysis seems quite thorough and overall conclusions make sense. I support its ultimate publication after appropriate revisions."

We thank Reviewer 1 for this positive assessment of our work.

"Specifically, I believe the major advantages of the theoretic framework should be better presented and clarified. This difficulty probably stems from the presentation of technical details, which are quite dense and sometimes difficult to digest. The parts that can demonstrate the utility of the formulation are the interesting results presented in Figure 4 and Figure 5. Here they show that the signaling length scale can serve as a predictor whether self-organized structures can emerge from a homogenous initial condition - a longer signaling length scale leads to more coordinated behavior. The result makes sense. But from the point of demonstrating a new framework, the authors need to better articulate

its advantages - is it primarily computational? Or can the framework make it easier to predict certain features that would be difficult for a typical method? Without addressing these questions (even in writing), the reader is left wondering why one would want to use this new theoretic framework.

In general, their presentation can probably be substantially simplified to better highlight key points."

We agree that more clear explanations will enhance our manuscript. Throughout our revised manuscript, we have tried to provide clearer explanations of our key results. Our key results are:

- We show, step-by-step, how complexity arises from simplicity. We use simple methods and simple components, but derive non-trivial results at the population level. Providing a unified, simple, and broadly applicable framework that quantifies concepts that are often described in mere qualitative terms (e.g., cell's "amount" of autonomy) by using experimentally accessible parameters is the most important point of our work. Our additional work on spherical cells and finite Hill coefficients now strengthen our claim that point-like cells with an infinite Hill coefficient capture many of the main features of real biological systems.
- By introducing the concept of "entropy of population", we can predict, based on just three molecular parameters (S_{ON}, K, L), the type of spatial patterns that can form and how spatially ordered this structure would be, all without having any knowledge of the initial ON/OFF state of every (or any) cell in the population. In this sense, the entropy of population is similar in spirit to the thermodynamic entropy and Shannon's informational entropy. We have added a section in the main text to explain this.
- Introduced the spatial clustering index I_M as a measure of spatial order (i.e., orderliness of patterns formed by ON/OFF gene expression in a field of cells) and the entropy of population.
- We define and use phenotype diagrams to geometrically represent the fundamental trade-off between autonomy and collectiveness.

"Other specific points
(1) Explanations of Figures 2 B and C are confusing. It took me multiple tries to flip between the figure and the main text to understand the presentation (I think). $\tilde{S}\_ON > \tilde{S}\_OFF$ is the criterion in defining ON cells and OFF cells, mathematically. This point has not been made very clearly in the text. Readers may feel confused about the statement: $S\_ON > 1$ there are only three combinations of φ_OFF and φ_ON."

We have now clarified this by adding the following sentences:

- In the paragraph above equation [1], we added, "R_{ON} is larger than R_{OFF}." This follows from the definition of our system.
- We deleted the sentence just before equation [5]: "Since $S_{ON} > 1$, we have three

possible combinations for the signs of φ_{OFF} and φ_{ON}".

- Then just after equation [5], we added: "The scenario in which $\varphi_{OFF} > 0$ and $\varphi_{ON} < 0$ cannot occur because the secretion rate of the ON cell (R_{ON}) is larger than the secretion rate of the OFF cell (R_{OFF}). Thus the concentration on the surface of the ON cell (S_{ON}) is larger than that of the OFF cell ($\tilde{S}_{OFF} = 1$). Thus $\varphi_{ON} > \varphi_{OFF}$ and hence we cannot simultaneously have $\varphi_{OFF} > 0$ and $\varphi_{ON} < 0$."

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*"(2) The authors demonstrate how the secrete-sensing cells could dynamically change from a disordered pattern to a highly ordered pattern. This is evident in the stripe pattern in Figure S7. However, the authors did not explain what factors are driving the cells to formulate stripe or island patterns. Does it have anything to do with initial cell distribution, or only the fraction of ON cells? How often can stripe patterns be generated? Currently the authors use a grid of 21*21 cells. If the grid size is increased to 35*35 cells, would ordered stripe patterns still emerge?"*

To obtain the stripe pattern in Figure S10 (Fig. S7 in the original manuscript), we ran many simulations by randomly distributing the ON and OFF cells over space with only two constraints:

- (1) 50% of the cells in the population are ON and the other 50% of cells are OFF (this is what we mean by "p = 0.5" in the figure caption) and
- (2) The cells are in the neighbor-induced simultaneous "activation-deactivation" region of the phenotype diagram (by having the values K = 61, S_{ON} = 30, and L = 0.7).

With these two conditions, we observed in many of our simulations (including with populations larger than 21x21 cells) self-organization of stripes (Fig. S10) and islands (in Fig. S11, shown for a larger population: 35x35 cells). Since these simulations are run with initial spatial distributions of ON and OFF cells that are random, we did not predict in which particular initial spatial distributions of ON and OFF cells lead to ordered spatial patterns. But instead, we can explain why certain spatial patterns are stable. A small island of ON cells will produce a small concentration of the signaling molecule on its boundaries whereas a larger island or a whole stripe of ON cells will produce a higher concentration of the signaling molecule on its boundaries. We show the values of the three molecular parameters (K, S_{ON}, and L), in which these spatial patterns are more stable than the others. Thus we are showing an important link between the topology of spatial patterns at the population level and the molecular parameters that define cell-cell communication.

Stated in other words, we can predict for which values of the molecular parameters (K, S_{ON}, and L) a population can generate and sustain stable and highly ordered spatial patterns. Given that in any realistic situation, an experimentalist would not necessarily have the full information about the ON/OFF state of every (if any) cell and how they are arranged in space, we believe that it is more important and useful to know when stable patterns can form based only on our limited knowledge of just a few molecular parameters (K, S_{ON}, and L). Our work provides precisely this insight through

the "entropy of population".

"(3) Figure 4C: Typo, signalling→ signaling?"

We previously used the Canadian/British spelling in our manuscript. We have now switched to the American spelling throughout our manuscript to conform to *Cell Systems*' style.

"(4) I don't completely understand the significance of the last paragraph in the results section that describes the mean-field model for mixed liquid culture. Some clarification on this point could better deliver the message that they were trying to convey."

We have rewritten this section to better convey our main idea. Our main idea here is that we want to understand how having ON cells clustered in one location to some degree (as measured by the spatial clustering index I_M) affects how the fraction of ON or OFF cells in a population changes over time (i.e., effects of spatial clustering on population-level dynamics). The mean-field model enables us to address questions such as how a small island of ON cells causes the fraction of ON/OFF cells to change over time in the entire population. To do this, we consider a "control" system: A uniformly mixed liquid culture of cells. There is no spatial organization of ON and OFF cells in this system. In fact, each cell would have a neighbor who's state randomly varies between ON and OFF over time (because cell would be moving in a liquid culture and randomly encountering their neighbors). Due to restrictions on the length of our manuscript, we have relegated the more detailed description of the mean-field model to the Supplementary text.

"(5) The conceptual framework in part stems from Youk's previous work (Youk & Lim). It would be interesting to apply their modeling analysis to their experimental systems, which may help illustrate the advantages of new theoretic framework. "

Indeed. But given that our current manuscript is focused on establishing a theoretical framework that's useful for our and other researchers' future experiments, we would like to postpone such experiments.

"(6) Please specify the condition of generating Figure S8: i.e., fraction of ON cells, initial cell distribution (do a and b start from the same initial cell pattern)?"

We have now clarified this in the caption for Fig. S11 (Fig. S8 in the original manuscript). In both (a) and (b) of Fig. S11, we initially have p=0.5 (i.e., 50% of the cells are initially ON while the other 50% of the cells are initially OFF). With this constraint, each cell in the population is randomly assigned to either the ON or the OFF state at the beginning

of each simulation. Then we run the simulations. Thus both (a) and (b) start with p=0.5, but the exact location of each ON and OFF cells will be different for both. So (a) and (b) start with different initial patterns. These images are representative images from the many simulations that we ran with p=0.5. Statistics from all these simulations are given in Figs. S6 and S7 (in terms of the final value of the fraction of ON cells (p) and of the spatial clustering index (I_M).) So these highly spatially ordered patterns shown in (a) and (b) accurately represent high spatial order we get when we initially have p=0.5.

> *"(7) I am surprised to see that this simple motif can generate well-organized islands and stripe patterns. I do realize that the biggest cell grid in your simulations is 35*35. I understand increasing the grid size will dramatically increase your calculation efficiency. However, even in Drosophila, within 4 hours of fertilization, there will be ~6000 cells in the cellular blastoderm. For some bacterial colony patterns, colonies are made of over millions of cells. How would you predict the final clustering index with large initial cell numbers? "*

Indeed, the larger the grid (number of cells) is, we would need more computational power. But we can reason, without running simulations for much larger sizes (our maximum size is 35x35=1225 cells), that since the signaling length L is short, areas or patches of islands that are distant from each other should be independent of each other. Thus we predict similar final clustering index for much larger populations.

> *"(8) They describe that they did not repeat the simulations for the negative feedback regulation since it would utilize the same theoretical formalism, however, I wonder how clustering index would change within the negative feedback."*

In our theory, the negative feedback generates similar values for the clustering index as the positive feedback because the values for K, S_{ON}, and L that are required to activate, deactivate, and simultaneously activate-deactivate are the same for both feedbacks. That is, the positive and negative feedbacks have identical values that define the boundaries between the different phenotypes in their respective phenotype diagrams. To be explicit, consider the "flip-flop" domain in the phenotype diagram for the negative feedback. The equivalent region for the positive feedback cell is the "bistable" region. The negative feedback cell would flip back and forth between ON and OFF states. If we add interaction between neighboring cells, some cells will "shift" their oscillation between ON and OFF from one time step to next. For example, if we have one OFF cell surrounded by ON cells, the signaling molecules from the ON cells will inhibit the OFF cell. This prevents the cell from flipping ON in the next time step and will instead stay OFF. Thus at the next time step, all the cells will have switched OFF (i.e., the neighbors would have flipped from ON to OFF). Another way to saying this is that looking at two time steps in the negative feedback is basically equivalent to looking at one time step in the positive feedback case. In the positive feedback, the "basal" state is to stay in the same state (bistability) and the neighbors that signal to the cell trigger the cell to change

its state. In the negative feedback case, the "basal" state is to change its sate and the neighbors signaling to it trigger the cell to stay in the same state. Apart from this difference, the quantitative values needed for activating and deactivating are the same. Thus the clustering index for both are similar as a function of L, K, and S_{ON}.

> *"(9) The authors claim that "Spatially disordered population of cells can dynamically form ordered spatial patterns due to a decrease in the entropy of population". This is not precise: "entropy of population" is a measure they introduced to quantify the degree of disorder. Its decrease is not the cause of spatial patterns emerging."*

We have added the section "Usefulness and significance of the entropy of population" (2nd last section before the discussion section).

Yes the Reviewer's correct in that we need to be more precise in distinguishing between a cause and a correlation. We have clarified this in the manuscript. The point we're trying to make is that without knowing the ON/OFF state of every (or any) cell in a population, we can precisely predict what kinds of spatial patterns can arise using the entropy of population. A key point here is that the connection between spatial order and the entropy of population is nontrivial. Although we may intuitively expect it, there is no rigorous apriority reason for expecting the inverse correlation between a high spatial order (measured by the clustering index) and the low entropy of population. Yet this is what we have uncovered. We now clearly explain this in our manuscript.

> *"(10) Section "Application of our general formalism: Population of an arbitrary size", second paragraph, last sentence: Should a cell's degree of autonomy be represented by the combined area of orange, yellow, and blue (not yellow) regions?"*

Yes the Reviewer's correct. This was a typo. We have now corrected it.

We thank Reviewer 1 for his/her positive overall assessment and insightful comments that have improved our manuscript.

Response to Reviewer 2:

We thank Reviewer 2 for his/her generally positive assessments and detailed suggestions, which enabled us to improve our manuscript. Remarks of Reviewer 2 are denoted in *italics*.

"In their manuscript, "Molecular contol of collective systems", Maire and Youk ask an important and interesting question about the control of molecular populations: can one characterize when a communicating population behaves collectively or individually as a function of some basic biophysical parameters and engineering design parameters? The research program is an ambitious one- and the manuscript makes progress towards to this important role."

We thank Reviewer 2 for this positive assessment.

"The approach employed by the authors is to use an "infinite Hill coefficient" approximation (almost identical in spirit to Dayarian, Adel, et al. "Shape, size, and robustness: feasible regions in the parameter space of biochemical networks." PLoS Comput Biol 5.1 (2009): e1000256 which should be cited) in combination with a "cellular point-source" model on a regular lattice to develop a physics inspired "toy model" of communicating populations. Using this simplified but insightful model, Maire and Youk are able to construct phase diagrams that show that there are different regimes of behavior for populations. In this, they show the basic idea underlying the earlier experimental paper of Youk and Lim can be expected to be quite general - communicating populations can transition from individualistic to collective behaviors."

We now cite Dayarian et al. 2009. We also thank Reviewer 2 for this positive assessment.

"The major deficiency of the manuscript, at least, as it currently stands is the toy model is full of extreme approximations that while making the calculations tractable, make it unclear how general the results presented here. While there is no doubt that there will be crossovers from more individualistic to more collective behavior, it is really unclear how sharp these will be in real systems and whether there is really a clean separation between these concepts as is implied by the toy model.

In order to convince me of that the results are as general as the introduction suggests, the authors would have to run much more sophisticated PDE like simulations where - cells are no longer point sources but instead have sizes comparable to inter-cell distances, the infinite hill coefficient approximation is relaxed, and the cells are no longer on a periodic regular grid but instead form some more realistic biofilm structure."

As all three reviewers have pointed out, the main goal and strength of our work is showing how *simplicity generates complexity* rather than using complex building blocks to build further complexity. For this reason, we have used regular lattice, infinite Hill coefficient, and separation of time scales in which we assumed that diffusion occurs much faster than the time required for regulating gene expression.

We agree with Reviewer 2 that we need to carefully justify these assumptions, explain when they are applicable to biological systems and when they are not, and importantly, show how our key results change if we relax some of our simplifications. Based on Reviewer 2's suggestions, we have now performed significant additional work to address these. In particular, we now include

1.) New results on a finite (not point-like) 3D spherical cells.

- **Added Figures 2D, 2E, and 2F:** Phenotype diagrams of an isolated spherical cell with radius R. We found that the phenotype diagrams remain qualitatively unchanged, compared to the point-like cells. This is true for isolated cell, basic unit, and for a population of *N* spherical cells.
- **Added the section "1. Three dimensional, spherical cell with a finite radius" in the Supplementary Information** (Pgs. 17-19) that describes all the additional calculations.
- **Added supplementary figures Figs. S1, S2, and S3** that show the phenotype diagrams of an isolated spherical cell, basic unit of spherical cells, and the main difference between the phenotype diagrams of *N* spherical cells and the phenotype diagrams of *N* point-like cells. As a result of adding these new figures, we have renumbered the supplementary figures from the previous version of our manuscript.
- **Refer to and summarize these throughout the main text, where appropriate.** Due to the limited space, we keep the discussions on spherical cells in the main text short and present only the summaries of the more detailed explanations that are presented in the Supplemental text. We refer the reader to the details in the Supplemental text.

Conclusion 1: *3D spherical cells with an infinite Hill coefficient have qualitatively identical phenotype diagrams as point-like cells. There are only minor quantitative differences that depend on the radius of the cells.*

2.) New results on finite Hill (not infinite) coefficients.

- **Added the section "6. Finite Hill coefficients" in the Supplementary Information** (Pgs. 27-31) that describes new analytical framework and computational algorithms that we developed to treat point-like and spherical cells with a finite Hill coefficient.
- **Added supplementary figures, Figs. S13, S14, S15, and S16**.
- **Added "Cells with a finite Hill coefficient" section in the main text** (just before the discussion section). Due to the limited space, we keep this section

short by summarizing the more detailed explanations that are given in the Supplemental text.

Conclusion 2: *Cells with a finite Hill coefficient have phenotype diagrams (for isolated cell, basic unit, population of N cells) whose main qualitative features are essentially identical to the main features of the phenotype diagrams for cells with an infinite Hill coefficient. Crucially, the algorithms, methods, and framework that we developed in the main text for cells with an infinite Hill coefficient still apply to cells with a finite Hill coefficient. The main quantitative difference is the appearance of a "continuum" region (i.e., in between "ON" and "OFF" regions) in phenotype diagrams. These regions have minimal areas for high Hill coefficients (e.g., n=2; considered to be high in many real systems), intermediate areas for medium Hill coefficient (e.g., n=1.5) and large areas for very low Hill coefficients n (e.g., n=1). We have now quantified the nature and the location of these continuum regions in phenotype diagrams (including for populations of N cells - Figs. S15 & S16).*

Putting Conclusions 1 & 2 together, we can now say that our results on the point-like cells with an infinite Hill coefficient capture the main features of more realistic, three-dimensional cells with finite Hill coefficients despite some quantitative differences. For this reason and because they are the easiest to comprehend for the general reader, we have kept all our results on the point-like cells with an infinite Hill coefficient in the main text and the main figures. The additional work suggested by Reviewer 2 allows us to put these results on a more solid ground. We thank Reviewer 2 for suggesting these additional studies.

We have not treated irregular lattices in our revised manuscript. Here we have followed the Editor's advice that it would be beyond the scope of our current work. But we nevertheless agree with Reviewer 2 that considering irregular lattice would likely lead to interesting results.

"Having said this, let me add that I think the manuscript is a good one and makes an important point. It is just that I would say that, as a theory/computational manuscript, right now the technical tools brought to bear on the problem are a little bit underwhelming. It is not clear what will happen when the manifold assumptions underlying the toy model are relaxed.

In summary, Maire and Youk have created an interesting toy model that suggests there should be transitions between individualistic and collective behavior. The model has the virtue of clearly demonstrating some important ideas within a simplified framework that captures many important details. Furthermore, the basic question asked by the manuscript is extremely important and there is more and more interest in addressing it. For this reason, I feel the manuscript is likely to be an important one - despite the current deficiencies."

We thank Reviewer 2 for this positive assessment. Regarding substantial additional work

that we have carried out, please see our comments below.

"Specific Comments:

1. "A sigmoidal function usually describes the cell's secretion rate and the reporter gene's expression level as a function of the signalling molecule's concentration. In many secrete-and- sense cells found in nature, a step-function closely approximates the sigmoidal function (Figures 1D and 1E) "

This is not necessarily true. The only experimental measures I know of this are two papers out of the Bassler lab- Long et al 2010 and Teng et al 2012), where multiple mutants were fit with Hill coefficient 1. For this reason it is important to ask what happens when the infinite hill coefficient approximation is relaxed."

We have now carefully investigated the effect of having a finite Hill coefficient. We have carried out the following additional work:

- **Added the section "6. Finite Hill coefficients" in the Supplementary Information** (Pgs. 27-31) that describes new analytical framework and computational algorithms that we developed to treat point-like and spherical cells with a finite Hill coefficient.
- **Added supplementary figures, Figs. S13, S14, S15, and S16**.
- **Added "Cells with a finite Hill coefficient" section in the main text** (just before the discussion section). Due to the limited space, we keep this section short by summarizing the more detailed explanations that are given in the Supplemental text.

  From these, we conclude that cells with a finite Hill coefficient have phenotype diagrams (for isolated cell, basic unit, population of N cells) whose main qualitative features are essentially identical to the main features of the phenotype diagrams for cells with an infinite Hill coefficient.

But there are important quantitative differences that we now explain in our manuscript. As Reviewer 2 correctly predicted in his/her earlier statement, the finite Hill coefficient leads to an appearance of a "continuum" region (i.e., in between fully "ON" and fully "OFF") in the phenotype diagrams. We have used a "phenotype score", which is a number between 0 and 1, to quantify this continuous spectrum (Figs. S15 & S16). A fully OFF cell gets a 0 while a fully ON cell gets a 1. For high Hill coefficients such as $n = 2$, the phenotype diagrams have nearly discontinuous boundaries between OFF and ON regions, just as in the cells with an infinite Hill coefficient. For biologically relevant, "intermediate" Hill coefficient such as n=1.5, the transition between OFF and ON regions in the phenotype diagrams are more continuous. For very low Hill coefficient ($n=1$), the continuum is quite broad.

Despite the appearance of this continuous region, the formalism that we have developed for cells with an infinite Hill coefficient (i.e., the signaling strength function $f_N(L)$, the method of focusing on "cell-I" and the concentration S_I that it senses, the method of constructing the population's phenotype diagrams starting from an individual cell) all apply to the cells with any finite Hill coefficient. The only difference is that the terms in these formulas are different. We have explicitly written down these formulas in the supplementary text.

"2. "For simplicity, we treat the cell to be point-like."

It is not clear this is a reasonable approximation, (when combined with way signaling profiles are treated as point sources with a single length scale -Thiel length- for decay) for any realistic cell population where inter-cell distances comparable to cell size. In particular, I expect the diffusion equation for these more realistic models to have many boundary layers and boundary effects that likely to be really important in a realistic system."

We used periodic boundary condition, which can simulate a closed tissue (closed sheet of cells). Even for some real biological systems with a cut-off boundary, our periodic boundary condition has shown to be applicable. An example is the recent work on a 2D sheet of hair follicles that secrete "distress signals" (Chen et al., *Cell* 2015). The macroscopic hair follicles (~100 μm in diameter) were also treated as "point-like" cells. Both assumptions led to theoretical predictions that matched the main results of the authors' experiments. Our additional work on spherical cells shows why treating cells (or even macroscopic hair follicles) to be point-like is an idealization that can still produce results that are relevant for real biological systems.

Following Reviewer 2's suggestion, we have now carefully investigated 3D spherical cells arranged in a 2D "tissue". Importantly, we have investigated populations in which the distance between the spherical cells could be any number (i.e., tissue of spherical cells that are touching each other or a tissue of spherical cells that are separated from one another by a great distance). As a result of these additional analyses, we have:

- **Added Figures 2D, 2E, and 2F:** Phenotype diagrams of an isolated spherical cell with radius R. We found that the phenotype diagrams remain qualitatively unchanged, compared to that of the point-like cells. This is true for isolated cell, basic unit, and for a population of *N* spherical cells.
- **Added the section "1. Three dimensional, spherical cell with a finite radius" in the Supplementary Information** (Pgs. 17-19) that describes all the additional calculations.
- **Added supplementary figures Figs. S1, S2, and S3** that show the phenotype diagrams of an isolated spherical cell, basic unit of spherical cells, and the main difference between the phenotype diagrams of *N* spherical cells and the

phenotype diagrams of *N* point-like cells. As a result of adding these new figures, we have renumbered the supplementary figures from the previous version of our manuscript.

- **Refer to and summarize these throughout the main text, where appropriate.** Due to the limited space, we keep the discussions on spherical cells in the main text short and present only the summaries of the more detailed explanations that are presented in the Supplemental text. We refer the reader to the details in the Supplemental text.

  From these, we conclude that 3D spherical cells with an infinite Hill coefficient have qualitatively identical phenotype diagrams as point-like cells with only minor quantitative differences that depend on the radius of the cells.

The reason that having spherical cells doesn't affect the main results is that our phenotype diagrams rely on the ratio between S_{ON} and S_{OFF}. While their values for a spherical cell are different from those of a point-like cell, the radius *R* and other factors affect S_{ON} and S_{OFF} in the same way (Fig. S1). Thus, the ratio S_{ON} and S_{OFF} is *independent* of the radius of the cell *R*. This means that for a spherical cell with a fixed radius *R*, if we measure all concentrations relative to S_{OFF} (i.e., unit in which $S_{OFF} = 1$), then we obtain exactly the same phenotype diagram for an isolated spherical cell as we do for an isolated point-like cell (Figs. 2E & 2F).

As for the basic unit of spherical cells and a population of *N* spherical cells, we again obtain a nearly identical looking phenotype diagrams as those of the point-like cells. We can use the same formalism as the point-like cells (i.e., the signaling strength function $f_N(L)$, the method of focusing on "cell-I" and the concentration S_I that it senses, the method of constructing the population's phenotype diagrams starting from an individual cell). But now the specific terms appearing in the formulas are different (explicitly written out in the Supplementary text). We used one simplification in our calculations for a population of *N* spherical cells: We assumed that in a population, each spherical cell senses the concentration at its center rather than on its surface. This simplifies our calculations because it avoids some "boundary layers" that the Reviewer may be referring to. But the main idea would remain the same as what we show in our additional work.

"3. "Since the concentration usually reaches a steady state much faster than the time taken for this averaging " --

This is another critique of the manuscript. There are many scales in the problem: time scales, density, length scales, concentrations. The approximations used in the problem assume specific things about the relationship between these scales but these assumptions are never stated explicitly. It is also not clear what happens when these assumptions are relaxed, hence the need for better simulations. For example, the text is interspersed with statements like:

> *"Instead of trying to find its exact value, let's assume that the Yi's are weakly dependent (i.e., almost independent) of each other, due to the exponentially decaying value of the concentration as a function of distance from the secreting cell"*
>
> *This is certainly not true for most cellular systems. Through out, assumptions should be stated clearly. This assumes extremely large degradation rates and small Thiele length (an assumption made implicitly through out the manuscript)."*

The following is our reasoning for assuming that concentration of signaling molecules reaches a steady state on the cell surface much faster than the time taken to regulate gene expression. First, for diffusible molecules, solving the diffusion equation exactly yields an exponential decay of the concentration over space (and in 3-dimensions, even a faster decay over space due to an exponential decay coupled to 1/r type decay, which we show in our additional analyses of spherical cells). The time-dependent, full solution to the 3D diffusion equation for a spherical cell of radius *R* that secretes a signaling molecule a constant rate η yields the following concentration *S* on the cell surface at time *t*:

$$S(R,t) = \frac{\sqrt{t}\eta}{\sqrt{\pi D}}\left\{1 - e^{\frac{-R^2}{Dt}} + \frac{R\sqrt{\pi}}{\sqrt{Dt}} erfc\left(\frac{R}{\sqrt{Dt}}\right)\right\},$$

where *erfc* is the complementary error function. We did not use any assumptions to obtain this exact solution. We note that the characteristic time (i.e., time required to reach steady-state concentration $R\eta/D$ on the cell surface) is determined by R^2/D because it has units of time and appears in the exponential and the *erfc.* We can put in biologically realistic values for *R* and *D* to estimate the time it takes for a typical cell to establish a cloud of the signaling molecule around itself whose concentration is at steady state. For example, in budding yeast the mating pheromone (α–factor) has *D* ~150 $\mu m^2/s$ and the radius of the yeast is *R* ~ 3 μm. We then have R^2/D ~ 0.1 seconds being the time required for the cell to establish a steady state concentration on its surface. In budding yeast, the genes that respond to the α–factor typically require about 1 hour to reach their steady-state expression levels, which is much longer than the 0.1 seconds required to create a steady-state diffusion cloud. Even if we consider a very large cell (*R* ~ 10 μm) and a very slowly diffusing molecule (*D* ~1 $\mu m^2/s$), the time required to create a steady state diffusion cloud around the cell is R^2/D ~ 10 seconds. This is still much faster than the typical time required for regulating gene expression. Thus we find that this reasoning justifies why we consider steady state of concentration to be reached much faster than the time required to regulate a gene.

One realistic way to delay the creation of steady state concentration (which undoubtedly happens in many real cells) would be to treat the secretion rate to stochastically vary between 0 and some other value over time (due to the fact that cells create proteins, including signaling molecules, in stochastic bursts rather than

continuously). But this is beyond the scope of our current work and of conventional methods for modeling secretion-diffusion systems.

We should also mention that our work allows the degradation rate to be any value (i.e., any decay length λ) and does not make assumptions about the value of λ. Our signaling length L is normalized by the intercellular distance "a_O", thus we do not think that it is the same as the "Thiele length" (or the Thiele modulus) that the Reviewer may be referring to. We observed the transitions between weak and strong neighbor communications for small (normalized) values of the critical signaling length L_c. This is due to the population size N that we have worked with (L_c depends on N and the geometry of the lattice). So we focused on small values of L, but it's not that we have assumed a fast decay of the molecule in any of our simulations or analytical results. Moreover, we have allowed the degradation rate γ and thus the values of L and λ to take on any possible values in our simulations and analytical calculations. In our revised manuscript, we have tried to better explain this and followed the Reviewer's suggestion to explicitly state what our assumptions are, whenever necessary. We apologize for any confusion that our insufficient explanations have caused.

"4. Regular lattices and boundary conditions

Things like regular lattices and boundary conditions will dramatically change any solution to the diffusion equation. These have to be treated with much more care than is done in the manuscript. One has to put no flux condition on irregular lattices (or really, non-point like particles for high density/low degradation rate regime)."

Our regular lattice is certainly an idealization and doesn't account for many biological systems. However there are numerous cases in which regular lattices have been shown to be good approximations of real fields of cells. For example:

1. A field of secreting-and-sensing hair follicles are arranged in a 2D regular polygonal lattice with a distance of ~0.15 mm between the centers of the follicles (Chen et al., *Cell* 2015).
2. The nuclei of the *Drosophila melanogaster* embryo at cycle 14 sit at the vertices of a triangular lattice of side length $a_O \sim 8.5$ μm (Gregor et al. *Cell* 2007)/
3. On nearly regular hexagonal lattice (like in our study), the secondary and tertiary pigment cells reside and surround the core ommatidium of the *Drosophila melanogaster*'s compound eyes (with the ommatidia themselves also arranged in a nearly regular polygonal lattice; Cagan and Ready, *Dev Biol* 1989) - Importantly, these cells and ommatidia can communicate with signaling molecules during development of the retina.

Regular lattice is not entirely unrealistic. But we agree with the Reviewer that we should mention the limitations of using a regular lattice. We now mention this in our manuscript.

In our work, we used periodic boundary conditions. This is equivalent to studying a

closed tissue (tissue that wraps around itself). Several other studies have shown that periodic boundary conditions can produce results that match experimental results. For example, Chen et al. *Cell* 2015 modeled their experimental system as 2D sheet hair follicles (large organs), in which each follicle was treated to be point-like secrete-and-sense "cells" and a periodic boundary condition was imposed on the whole sheet of follicles. This model produced results that matched their main experimental findings. However, there would be cases where periodic boundary condition wouldn't capture the key dynamics. But for the very large sheets of cells treated in our model (i.e., where the diameter of the field of cells is larger than the signaling length L), our main results wouldn't change qualitatively and would change only quantitatively if we switch to a different boundary condition. In particular, with an absorbing boundary condition, the concentration of the signaling molecules received by a cell would decrease as the cell is nearer to the edges of the lattice. In other words, the value of $f_n(L)$ would be lower for the cells in the edges of the population than those in the middle (for periodic boundary condition, $f_n(L)$ is the same for every cell, which simplified our calculations). In this case, we could say that the cells at the edges of the population would be more autonomous and that the cells in the bulk are more dependent on other cells. Depending on the parameter values, this "edge effect" could make the population less collective (measured by the areas in the phenotype diagrams) due to the cells in the edges interacting less with the other cells in the population. We now put this comment in the supplementary information section.

But more importantly, the regular lattice is the simplest and the first step towards quantitatively understanding cell-cell communication among the secrete-and-sense cells over space and time. Following the Editor's advice to us, we have not treated irregular lattices in this work. However we agree with the Reviewer that doing so would be interesting and we hope to pursue this in a future work (namely when "defects" arise in a lattice of cells).

> *"5. "they are in the "activation-deactivation" region (Figure 5C: white region in phenotype diagram), then a spatially disordered population of cells (i.e., IM ~ 0) has a higher chance of evolving into a population with spatially ordered patterns (i.e., IM closest to 1) than if the cells were in the activation region or the deactivation region (Figure 5C: compare the three graphs of IM) (also see Figures S3-S7)."*
>
> *I do not understand why I need a quantitative metric to understand this result. In general, the metric suggested here does not help me much quantitatively. Its not clear that its much more than a qualitative description."*

Some of these results are indeed intuitive but they hinge on being able to quantify the qualitative notions of "spatial order of cells". To be able to say that a population is spatially disordered or ordered, we need to be able to quantify this often qualitatively described trait. Our point is that if we don't have a quantitative measure (metric) for

spatial pattern formed by gene expression in cells, then we cannot properly talk about it just as we cannot talk about how autonomous or collective cells are unless we provide a plausible and measurable definition of the "amounts" of autonomy and collectiveness. Such quantification of notions that are conventionally only qualitatively described (at least when it comes to gene expression) is a central theme of our work.

Practically, the spatial clustering index I_M allowed us to run thousands of simulations, and then in a rigorous and unbiased way, put a number between 0 and 1 to the "spatial order" formed by the gene expression (ON/OFF) in a field of cells. In this way, we found that the metric I_M yielded results that match our intuition. Thus we posit that this is at least one sensible definition of spatial order. There are likely other definitions but that may be applicable to gene expression. Our I_M, which is closely related to the Moran's *I* used in epidemiology, is a good first step and also one of the simplest metrics that we can use.

We thank Reviewer 2 for his/her generally encouraging and positive assessments. We also thank Reviewer 2 for his/her insightful suggestions. We have made significant revisions based on his/her comments.

Response to Reviewer 3:

We thank Reviewer 3 for his/her positive assessment and valuable suggestions that have enabled us to improve our manuscript. Remarks of Reviewer 3 are denoted in *italics*.

"The authors present in their manuscript 'Molecular control of cellular collectives' a theoretical framework for multicellular behavior based on their recent (excellent) work on secret-and sense cells. They show that spatially fixed communicating cells can couple their behavior, which shifts behavior from single cell to collective behavior. This may also give rise to the formation of patterns.

The work is well done and richly illustrated. I also like that every mathematical procedure is described in the supplemental information very detailed. Expanding the secret-and-sense cell to a spatial situation is in general very interesting, and I felt that the authors have done a nice job of connecting disparate approaches in an elegant way. While each of the pieces may not be entirely novel, I believe that the manuscript has a whole does fills an important role in elucidating how the simple 'sense-and-secrete' ideas can in in principle be relevant to pattern formation. I therefore believe that this manuscript is appropriate for Cell Systems if the below concerns can be addressed."

We thank Reviewer 3 for this positive assessment.

"Main concerns to be addressed:
- My primary technical concern is that Equation [2] is the solution for a diffusion-degradation system in 1D, but Eq. [1] has to be valid for 2D with diffusion operator in polar coordinates. In particular, I think that the solution may have both an exponential and power law falloff. Although important, I don't think that this actually changes the conclusions of the paper in a dramatic way."

We apologize for the confusion that our insufficient explanation has caused.

Equation [2] is actually the solution to a 2-dimensional diffusion equation with degradation and secretion terms (Equation [1]) for a point-like cell. It indeed has the exponential fall-off term, $\exp(-r/\lambda)$ that the Reviewer mentions. But in addition, our solution is valid for a 2D diffusion operator in polar coordinates with a rotational symmetry (i.e., A point-like cell secreting in the same way in all directions, so there's nothing special about watching the cell from the East versus from the North, or from any other direction). This radial symmetry holds for an isolated cell (Equation [2]) but breaks down when we add more cells to the lattice. We take care of this broken symmetry by

summing up the 2D solution (Equation [2]) for each cell, invoking the superposition principle obeyed by the diffusion equation.

We did not clearly explain this before. In our revised manuscript, we now explicitly state that our solution is for 2D (not 1D) (i.e., we added "two-dimensions" in the sentence just before equation [1] and also in the sentence just before equation [2]).

"- Even if the signaling molecule doesn't degrade (gamma = 0) the concentration at the cell surface shouldn't go to infinity. Instead, the concentration at the cell surface should scale as D/R, where R is the radius of the cell. The concentration of the signaling molecule then falls as 1/r to some power that depends on the dimensionality (rather than exponentially). My suspicion is that this limit of a long-lived signaling molecule is probably more relevant for the kinds of situations that the authors are interested in."

We apologize for the confusion that our insufficient explanation has caused.

In our solution (Equation [2]), the concentration actually remains finite at r = 0 (cell surface). When λ is non-zero (i.e., in the realistic scenario in which the molecule will eventually degrade), then equation [2] yields S_O as the concentration on the cell surface (r = 0). S_O is equal to either 1 (if the cell is OFF) or equal to S_{ON} (if the cell is ON). As we take the limit of the degradation rate γ approaching zero (i.e., the limit of the molecule being permanently stable), λ approaches infinity but our solution still remains finite at r = 0 because the exponential term approaches 1. That is, the exponential decay gradually becomes less pronounced as the molecule becomes more stable (γ approaching 0).

- In the revised manuscript, we now explicitly state that our solution remains finite by adding the sentence: "Note that this solution remains finite even at r=0 (on the surface of the point-like cell).", a few sentences below equation [2].

But the Reviewer is correct in that a 1/r fall-off is expected, except that this would be for a cell that has a finite radius instead of being point-like. We have now included new results on a three-dimensional spherical cell with a finite radius R in our revised manuscript (please see our next comment). This solution also doesn't blow up at the cell surface because now r cannot be zero (minimum value of r is the non-zero radius of the cell).

"- Related to the previous point, the assumption that the cell is a point seems to cause problems, because then the concentration at the cell surface goes to infinity. The authors sidestep this by normalizing all the concentrations, but I am concerned that some readers will get confused. Also, I think that this choice may influence how the dynamics in the spatial setting will play out, since the relative importance of self versus neighbor signaling will depend upon the ration of the cell radius to the inter-cell distance."

Despite treating the cell to be point-like, our solution does not go to infinity at r=0 (the place where the cell is). It remains finite. We agree with the reviewer that this is confusing. Thus we now explicitly state this in our manuscript

While mathematically there's nothing wrong with treating a point object that secretes molecules, there are limitations. We therefore address this in our revised manuscript. Specifically, we now compute the results for a spherical (3D) cell with a finite, non-zero radius *R*. The main finding of this additional work is that increasing the radius of the cell decreases the steady-state concentration at the surface of the cell. Thus the phenotype diagram would "shift" towards a lower value for S_{ON}. This is equivalent to decreasing the secretion rate R_{ON} of the ON cell. For more details, please see our comments to Reviewer 2 on our additional work on spherical cells.

> *"- The authors should also say that they are assuming that only the 'cloud' of signaling molecule is being considered. In many situations the signaling molecule builds up over time, meaning that the concentration cannot be described by equation [6] but instead will be time-dependent."*

We now reiterate (after Equation [2]) that we are only considering a 'cloud' of signaling molecules whose concentration reaches steady state faster than the cell has the time to regulate its gene expression. The Reviewer is correct in that this cloud would build up over time. In our analyses, we assume that the time needed to build up this cloud is much shorter compared to the time required for adjusting gene expression. This is realistic for many biological examples. As an example, the time-dependence of the "cloud" is given by the full solution to the 3D diffusion equation for a spherical cell of radius *R* that secretes a signaling molecule a constant rate η yields the following concentration *S* on the cell surface at time *t*:

$$S(R,t) = \frac{\sqrt{t}\eta}{\sqrt{\pi D}}\left\{1 - e^{\frac{-R^2}{Dt}} + \frac{R\sqrt{\pi}}{\sqrt{Dt}} erfc\left(\frac{R}{\sqrt{Dt}}\right)\right\},$$

where *erfc* is the complementary error function. This reaches a steady-state concentration of $R\eta/D$ after a sufficiently long "characteristic time". The characteristic time is determined by R^2/D because it has units of time and appears in the exponential and the *erfc.* We can put in biologically realistic values for *R* and *D* to estimate the time it takes for a typical cell to create a steady-state diffusion cloud around itself. For example in budding yeast, the mating pheromone α–factor has approximately D ~150 $\mu m^2/s$ and *R* ~ 3 μm. We then have R^2/D ~ 0.1 seconds being the time required for the cell to establish a steady state concentration on its surface. In budding yeast, the genes that respond to the α–factor typically require about 1 hour to reach their steady-state expression levels, which is much longer than the 0.1 seconds required to create a steady-state diffusion cloud. Even if we consider a very large cell (*R* ~ 10 μm) and a very

slowly diffusing molecule (D ~1 $\mu m^2/s$), the time required to create a steady state diffusion cloud around the cell is R^2/D ~ 10 seconds. This is still much faster than the typical time required for regulating gene expression. Thus we find that this reasoning justifies why we consider steady state of concentration to be reached much faster than the time required to regulate a gene. So assuming that the steady state concentration of the cloud is realized before the cell has a chance to react (i.e., change its gene expression) is internally consistent within the framework of cells secreting at a constant rate. Real cells, however, may secrete in "bursts" (due to bursty production of proteins). This would be more realistic, lead to much slower build up of the cloud. But we feel that this is beyond the scope of our current work (we would need to start with a diffusion equation with a stochastic secretion term).

As another way to get a slower build up of the cloud, we have included additional work in which we studied cells with a finite Hill coefficient (i.e., not a step-function regulation of ON and OFF). For more details on this, please see our comments to Reviewer 2 on our additional work on cells with a finite Hill coefficient.

> *"- The spatial model is basically an Ising cellular automaton model (I think they should also mention and cite it) which is known to give rise to pattern formation and has also been used in the context of cellular and developmental biology in the past (Ermentrout, G. B. & Edelstein-Keshet, L. Cellular Automata Approaches to Biological Modeling. J. Theor. Biol. 160, 97-133 (1993))."*

We now mention the Ising cellular automaton model and cite the paper by Ermentrout & Edelstein-Keshet. (also Hopfield, *PNAS* 1982).

> *"- Regarding the flip-flop regime: The oscillations described in the manuscript are clearly possible formally, but is there an example of this actually happening? It seems that there would have to be a significant delay. I found the argument about timescales to be a bit funny somehow, as I felt that there were too issues. One is whether there is a separation of timescales and another of whether the cells are operating logic-wise in discrete or continuous time. These are different things and can lead to different conclusions."*

Indeed the Reviewer is correct that (1) the separation of time scales and (2) strict binary logic gates without the separation of time scales can both cause the negative feedback circuit to produce the oscillations. In our case, the oscillations arise primarily due to reason (1). Namely, we are assuming that the time taken for the cell to secrete the signaling molecules is shorter than the time that the cell takes to determine the concentration outside it, and then respond by changing the expression of the gene that encodes the signaling molecule and the reporter gene (Fig. 1C). This is why we have used discrete time steps to study the ON/OFF dynamics. In this formalism, the cell

cannot inhibit itself (i.e., switch from ON to OFF) in real time but postpones its inhibition to the next time step instead. This is the reason for the oscillations.

A potential signaling pathway that might exhibit this "flip-flop" switch is the autocrine signaling of insulin in the β-cells of the pancreas. In the islets of the Langerhans in the pancreas, the β-cells secrete and sense insulin through a self-regulated negative feedback. Namely, the β-cells decrease their rate of secreting insulin as they sense a decreasing concentration of extracellular glucose. The decrease in glucose occurs due to an increase in the concentration of extracellular insulin. Thus an increase in concentration of extracellular insulin causes the β-cells to decrease their secretion rate of insulin. To see the potential "flip-flop", one has to measure self signaling (Fig. 1B) and neighbor signaling (Fig. 1B) among the β-cells with a single cell resolution in intact islets. But such measurements have been challenging.

"Minor concerns:

- The increase in population and signaling strength in Fig. 4 is basically equal to increasing cell density and receptor density in their previous work. The spatial setting in this section can therefore perhaps be described more succinctly."

We have tried to explain this as succinctly as we can. But since we do not explicitly treat the abundance of receptor in our framework (but it's implicitly taken into account as a factor that affects the activation threshold *K*) and the previous work (Youk and Lim, 2014) dealt with "well mixed" configuration but not the lattice of spatially fixed ON/OFF cells with definite locations as we now do in our manuscript, we feel that it's important make this explanation clear.

"- The authors should be explicit that 'neighbors' in equation [6] refers not just to the immediate neighbors but rather to all the other cells in the population."

We now explicitly mention that the "neighbors" in equation [6] refers to all the other cells in the population and not just the nearest neighbors (we added the last sentence in the paragraph that follows equation [6]).

"- The authors state that the Ising type mechanism of pattern formation may be relevant in developmental biology because it allows to 'generate defined spatial patterns'. It is true that Ising models give rise to patterns but the patches are randomly distributed in space. But for the development of lets say an embryo one needs a specific cell type at a specific location. The Ising model probably does not provide that. I suggest that the authors weaken this statement."

Indeed. We have now weakened and clarified our statement. The Reviewer is correct in that our patterning mechanism cannot guarantee that, for example, a patch of ON cells will develop at an exactly predefined location in space. Our claim is that a random spatial distribution can generate ordered spatial patterns, but that it does not guarantee where in space a *particular* ordered pattern will arise. In our model, the exact location is determined by the initial locations of ON and OFF cells.

The question then is what sets up the initial patterns of the cells that are necessary to lead to the stripes or islands. Our model takes the initial ON/OFF state of the cells as a given but in many biological systems, this would not necessarily be the case. But if there would be another (additional) mechanism that sets up the initial patterns, then our model can be coupled with this additional mechanism to produce highly ordered spatial patterns. A broader way of saying this is that our Ising-like model generates defined spatial patterns modulo translation in space. So coupled with another mechanism that fixes the translation of the whole pattern, our model would provide how specific cell type (ON/OFF) would arise at a particular location in space, which could be relevant for developmental systems.

- We have now clarified this point in our revised manuscript by adding in the 2nd last paragraph: "But the caveat is that the exact location of the spatial patterns in the field of cells is determined by the initial locations of the ON and OFF cells. Thus if another mechanism sets up a particular initial pattern, which can be spatially disordered (i.e., $I_M \sim 0$), cell-cell communication among the secrete-and-sense cells can take over and generate highly ordered spatial patterns."

"- In line with the last point in the manuscript the secret-and-sense type of pattern formation in compared with the Turing mechanism. It is even stated that cells in the activation-deactivation region could provide the activation-inhibition framework and thus cause Turing like patterns. I disagree in two ways: A striking feature of Turing patterns is that for fixed boundaries the resulting patterns look always the same independent of the initial conditions, this is not true for an Ising model. Moreover in a Turing mechanisms there is not just activation and deactivation but the activating molecule is also activating the production of the inhibitor. This is not true for the secret-and-sense cells."

The Reviewer is absolutely correct about the two features of the Turing patterning mechanism. When we say "Turing-like" patterning in our manuscript, we do not mean that we can exactly map our system to the original Turing patterning formulation in a one-to-one manner. We now explicitly state that the patterns that we observe in our work are not due to the Turing patterning mechanism.

But we still invoke a discussion of the Turing patterning mechanism in our manuscript. Our main point here is to encourage experimentalists and theorists to think about how we can recast the original Turing's formulation of patterning, which involved diffusing molecules with the properties that the Reviewer mentions but did not involve cells and genes, in a modern framework that does involve cells. Such "Turing-like

patterning" (but not exactly the "Turing patterning"), in which there is still a notion of activation and deactivation (but not satisfying the criteria for exact Turing patterning that the Reviewer correctly points out), is what we want to highlight. Recent experimental work such as Economou et al. "Periodic stripe formation by a Turing-mechanism operating at growth zones in the mammalian palate" *Nat Genet* (2012), begs the practical question of how we can reformulate Turing's original mechanism in a "Turing-like" mechanism that involves cells. We believe that our work provides some insight into this process.

In the end, we agree with the Reviewer that we need to weaken some of our claims and clearly state that our formulation is not equivalent to that of Turing's, and that we are simply trying to understand how something like the Turing patterning mechanism could be reformulated in terms of cells (and thus necessarily deviating from the original formulation). We have done our best to make this clear by rewriting the section on Turing patterning to make sure that we are not overstating our results.

"- equations 4 and 5 are only true for positive feedback?"

Equations 4 & 5 are true for the negative feedback as well (i.e., they still mark the boundaries between the different phenotypes of a cell with the negative feedback, shown in Fig. 2C). We now clearly state this in the manuscript, just after introducing Equation [5].

"- The negative feedback is first described (with figure) and than never mentioned again. The authors could even remove discussion of negative feedback, or at the least reiterate to the reader that the spatial patterns discussed at the end of the manuscript only result from the positive feedback."

We followed the Reviewer's advice and have reiterated in our revised manuscript that the spatial patterns that we study are only from the cells with the positive feedback.

"- I do not really understand the use of the entropy of population. I mean it is true that the bigger the correlation length in the system the less equilibria configurations exist. But what information does this really add? I feel that giving the correlation length directly might be more useful."

The correlation length is useful and important. But it does not tell us about the total number of stable spatial patterns that a population can form. We sought out one number that could give us such a "global" picture, which the correlation length couldn't convey. This leads us to the entropy of population.

- We have added the section "Usefulness and significance of the entropy of population" (2nd last section before the discussion section) to clarify its meaning. The section reads as follows:

"As seen above, the entropy of population σ enables us to measure how many spatial patterns can be stably sustained in a population even if the only information we have about the population are the values of the three molecular parameters, S_{ON}, K, and L without knowing anything else. Crucially, without knowing anything about the initial ON/OFF state of every or even any cell in the population, the entropy of population allows us to still predict precisely how many spatial patterns can arise in the population and how likely it is that these patterns are spatially ordered (through the relationship between σ and the spatial clustering index I_M). Being able to predict a population-level property without having detailed information about the state of any individual cell makes the entropy of population similar in spirit to the thermodynamic entropy (Landau and Lifshitz, 1980) and the Shannon's informational entropy (Shannon, 1948), both of which quantify a systems-level property without having information about the detailed microstate of the system. Along these lines, our results above revealed that if the cells have a set of values for (K, S_{ON}, L) that lead to a high entropy of population, then the population of such cells likely have a low spatial clustering index I_M. Thus the entropy of population allows us to predict how likely the expression level of a gene (e.g., ON/OFF) in each cell in a population would form a spatially ordered pattern, in cases where we cannot experimentally measure the expression levels of a gene in any cell in multicellular systems such as a tissue or a biofilm. Intriguingly, this connection between the entropy of population and spatial order is reminiscent of the link between the thermodynamic entropy and the amount of disorder in a physical system, and also of the link between randomness of information in a message and the Shannon informational entropy. It may be fruitful to investigate if there are deeper connections between Shannon's entropy and the entropy of population, given that both deal with how much information is accessible to an experimentalist about a particular system."

"- The cluster index is defined for a 2D plane of a given size. However, the authors' system is (probably?) periodic, but the cluster index does not take that into account e.g. two positions at the left and right boarders of the plane are regarded as very far away in estimating the cluster index but because of the periodic borders they might be immediate neighbours."

Indeed we used the periodic boundary condition for all our calculations except in computing the clustering index. We now explicitly state and explain this in the "Definition of clustering index" section in the "Theoretical methods" section. Our rationale is that the clustering index is purely geometrical quantity (e.g., it measures how spatially clustered ON cells are to each other). If we used the periodic boundary condition for this, then every pair of cells would have two separation distances: (1) short distance and (2) long distance (e.g., going out the left end and then coming back from the right end of the field

of cells). To avoid arbitrarily choosing one of the two distances for each pair of cells and to simplify our calculations, we treated the field of cells to have hard edges (not connected edges) and computed the clustering index with these hard boundaries. This gives us clustering index that would be different from the clustering index computed under periodic boundary condition for some scenarios (e.g., particularly when a pattern is connected through the edges of the field of cells) but our main qualitative conclusions about spatial order/disorder would remain unchanged. We have now clarified this in the revised manuscript (in the "Definition of the clustering index" subsection of the "Theoretical methods" section. It's a subtle but important point. We thank the Reviewer for pointing this out.

We thank Reviewer 3 for his/her positive overall assessment and insightful comments that have improved our manuscript.

After revision and seeing our response to their comments, the reviewers responded with the following comments:

Reviewers' comments:
Reviewer #2:
Given the parameters of the editor, I am happy with the changes and new simulations run by the authors. They have addressed many of my concerns. As I stated in my previous review, I think the manuscript and approach are a good one and I recommend publication.

Reviewer #3:
The authors have addressed my concerns. I believe that this manuscript provides a significant conceptual advance that will be of interest to a wide range of authors, and I therefore recommend publication in its current form.