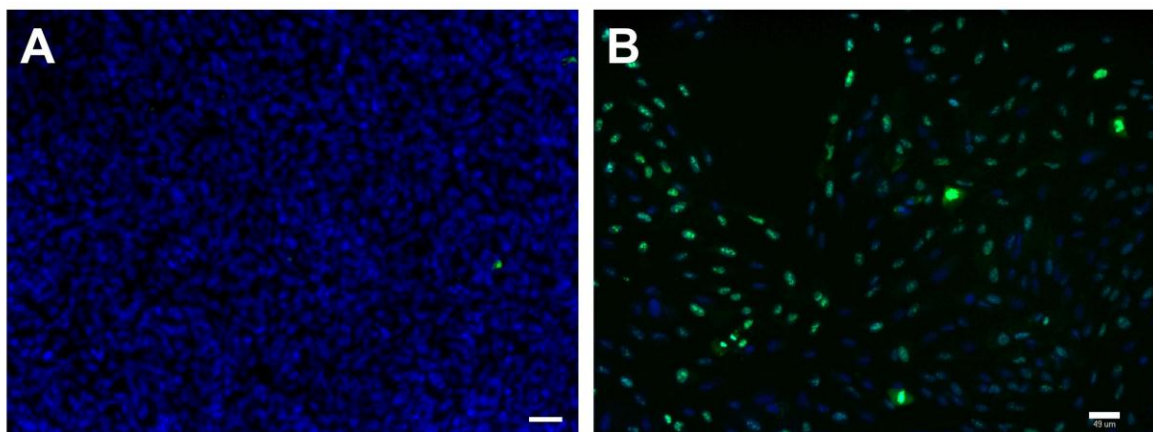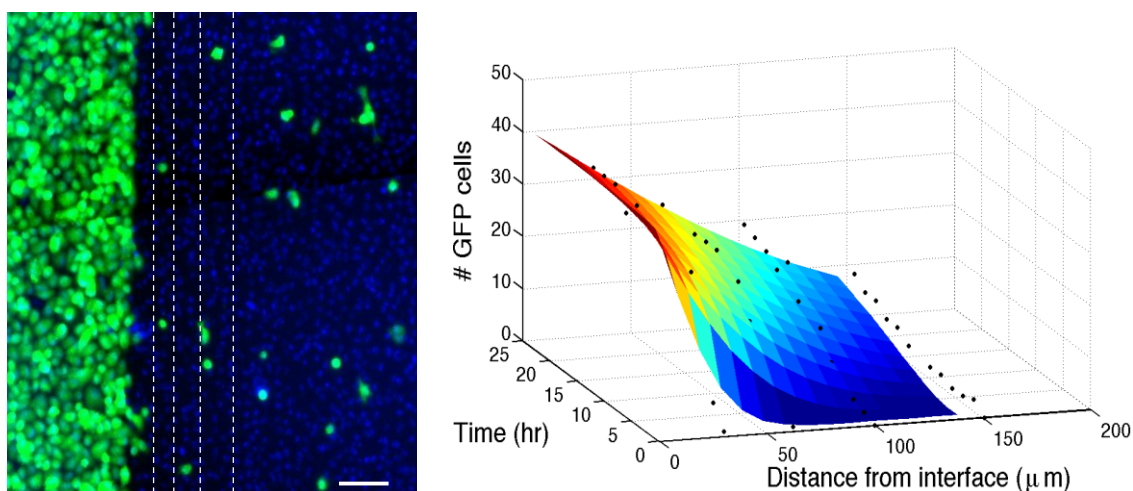## Supplementary Information



**Figure S1**. Cell proliferation in confluent sheets of ARPE-19 cells. *A –* Confluent sheet of ARPE-19 stained for Ki67 24 hours after initial cell seeding. 0.006±0.005% of cells were Ki67^{+}. *B –* Sub-confluent ARPE-19 cells stained for Ki67 24 hours after initial cell seeding. 78±4% of cells were Ki67^{+}. *Blue –* **DAPI;** *Green –* **Ki67.** The scale bar is 50 μm.

**Figure S2.** Estimating the random walk diffusion coefficient of cell migration.

*A -* The interface between eGFP-ARPE-19 cells and ARPE-19 cells used to estimate diffusion. A patterned co-culture of eGFP and wild type cells was prepared on transwell inserts as previously described [1]. The interface between the two cell types was imaged every 2 hours for duration of 24 hours. At each time point the number of eGFP cells on lines 30, 60, 100 and 150 μm away from the initial interface (dashed lines) was manually counted and used to model the random walk motion of cells. *Blue* – DAPI; *Green* – CFDA-SE. *B -* Fitting of the experimentally determined number of eGFP cells (filled circles) at indicated times and distance away from the initial interface (C(x,t)) to the finite width random walk diffusion equation.
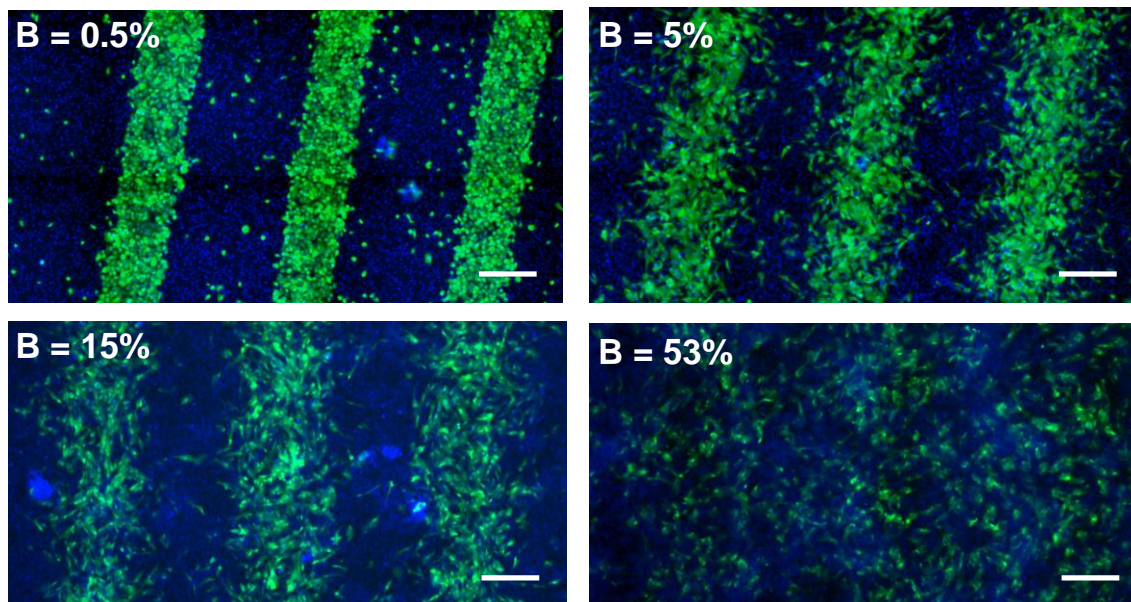
**Figure S3.** The pattern distortion associated with different levels of blurriness.

A co –culture consisting of 200-µm wide eGFP+ cells surrounded by wild type cells was generated as previously described [1]. The patterns were allowed to disrupt to different levels after which the cells were fixed and stained with DAPI. The coefficient of blurriness (**B**) was calculated as described in the materials and methods. The scale bar is 200 µm in width. *Blue* – DAPI; *Green* – CFDA-SE

**Table S1.** Comparison of experimental and predicted B values for patterns of different widths with or without blebbistatin.

| | 100 µm | | 200 µm | | 300 µm | |
|---|---|---|---|---|---|---|
| | with blebbistati n | no blebbistati n | with blebbistati n | no blebbistati n | with blebbistati n | no blebbistati n |
| **Experimental B, %** | 0.46±0.04 | 0.5±0.06 | 0.24±0.02 | 0.38±0.14 | 0.09±0.05 | 0.18±0.08 |
| **Predicted B, %** | 0.56 | 0.63 | 0.24 | 0.34 | 0.08 | 0.15 |
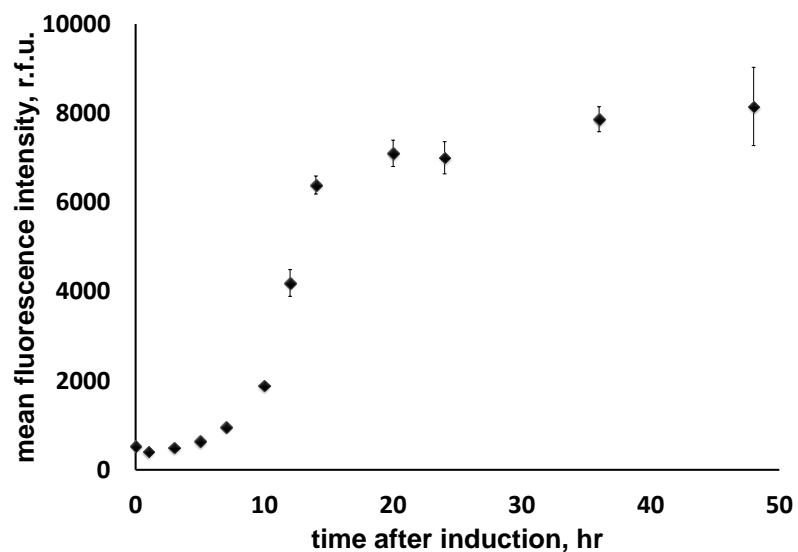
**Figure S4.** The induction dynamics of the Tet-Express gene expression system.

Tet-Express-mCherry-ARPE-19 cells were exposed to the Tet-express inducing agent. At the indicated times after the addition of the inducing agent, the cells were harvested by trypsinization, fixed, and their mCherry content quantified by flow cytometry. $n=3$, error bars represent standard deviation.
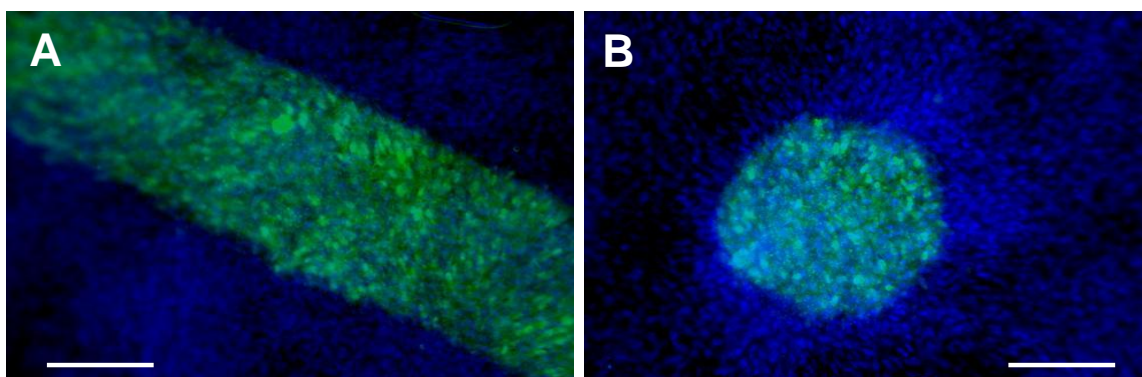
**Figure S5.** Patterning cell sheets with a fluorescent dye using Parafilm membrane inserts.

Transwell inserts with parafilm membranes were prepared, as described in the main text, and seeded with ARPE-19 cells at confluence. CFDA-SE was placed in the bottom chamber of the transwell system and incubated for 20 min. The dye and parafilm insert were then removed, samples fixed and stained with DAPI. *A -* a stripe pattern of CFDA-SE delivery was imposed by cutting a rectangular piece of parafilm out. *B* - a circular pattern of CFDA-SE delivery was imposed by making a hole in the parafilm insert using a 26G blunt-end needle. *Blue* – DAPI; *Green* – CFDA-SE. The scale bar is 200μm in size.
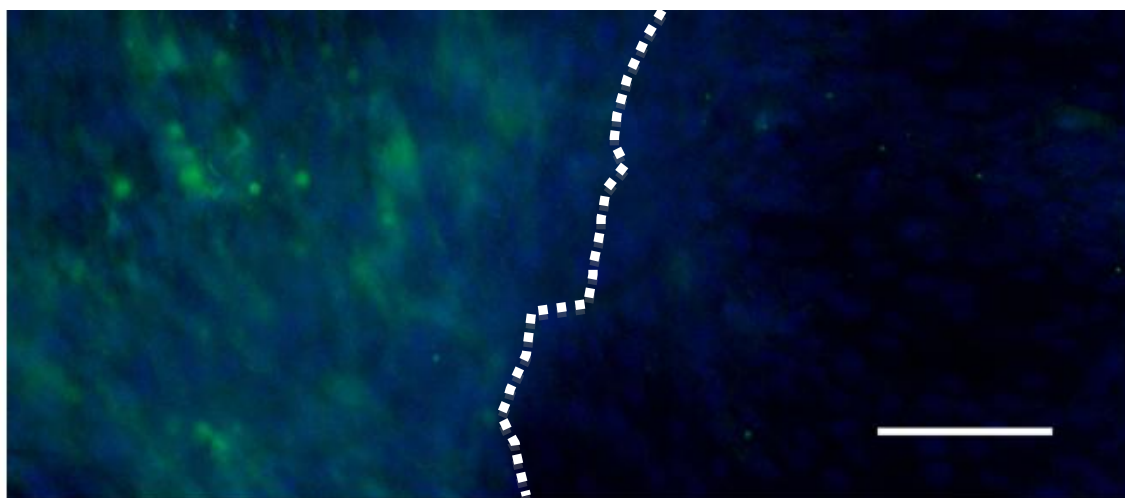
**Figure S6.** Lack of doxycycline transport between neighbouring cells.

A patterned co-culture of Tet-on-eGFP cells previously cultured in the presence of doxycycline (left) and Tet-on-eGFP cultured in the absence of doxycycline (right) was prepared as previously described by a method that minimizes the intermingling of two cell populations [2] and cultured for 6 days in doxycycline-free medium. The cells were fixed and stained with DAPI. *Blue* – DAPI; *Green* – eGFP. The scale bar is 200μm in size. The lack of significant eGFP expression in the cells on the right was interpreted as a lack of significant transfer of doxycycline between neighbouring cells.
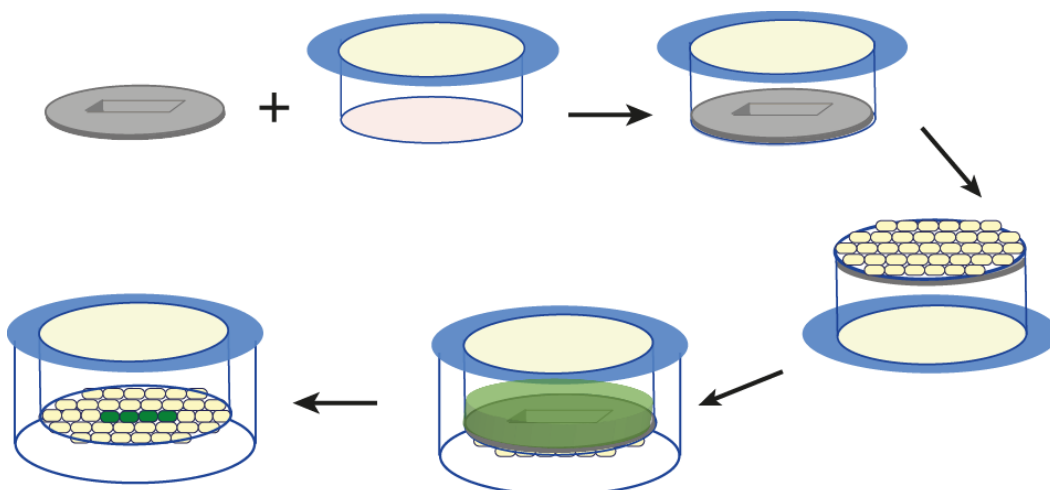
**Figure S7.** Schematic of adapted parafilm method for use with Tet-Express system. The side of the membrane insert on which the cells were seeded was reversed compared to the Tet-on patterning system. This was necessary due to formation of aggregates upon preparation of the inducing agent for the Tet-Express system.
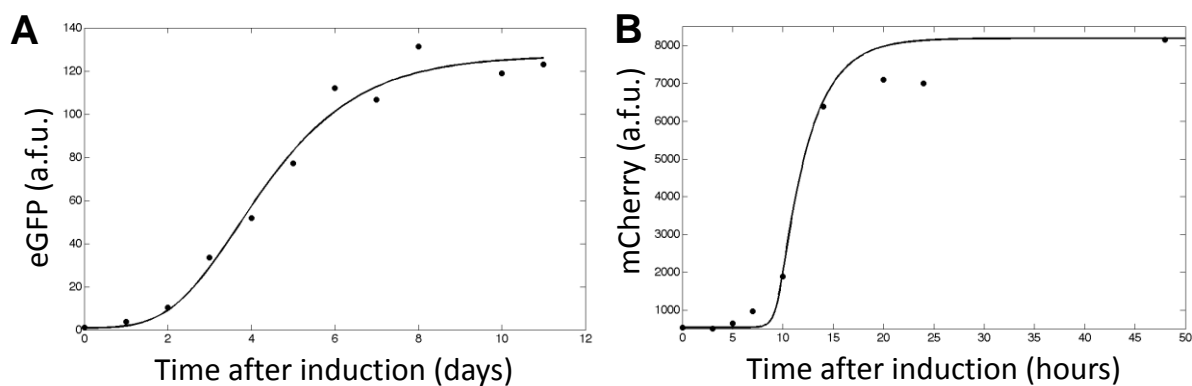


**Figure S8.** Experimental data (filled circles) and corresponding curves (solid lines) fitted using the model for the Tet-on (**A**) and Tet-Express systems (**B**).
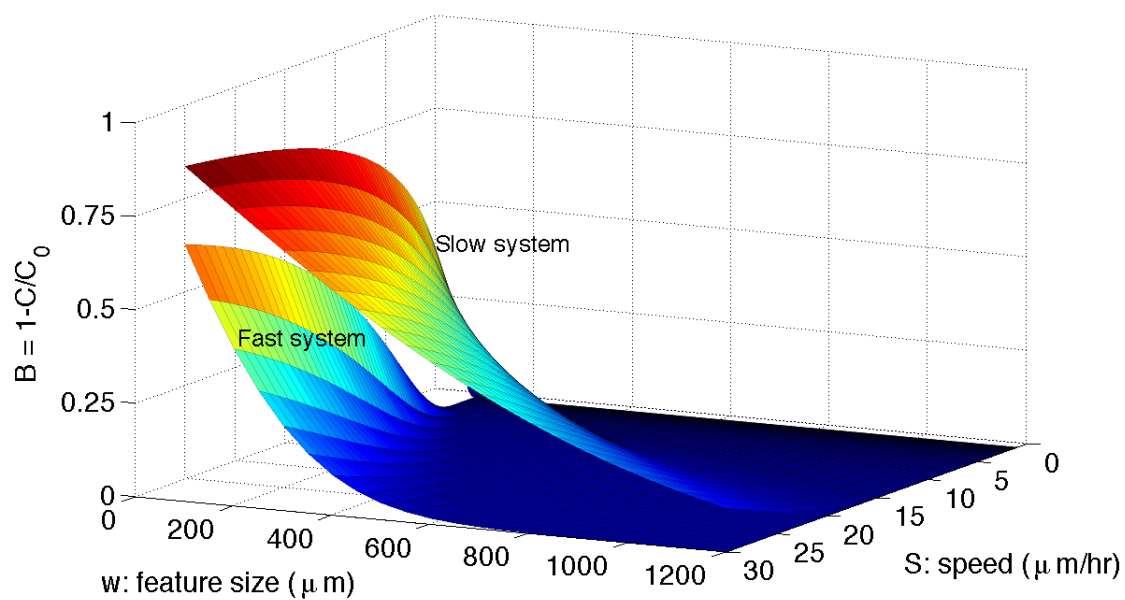
**Figure S9**. Comparison of blurriness levels expected for Tet-on (slow system) and Tet-Express (fast system) for different values of cell speed and different pattern feature sizes.
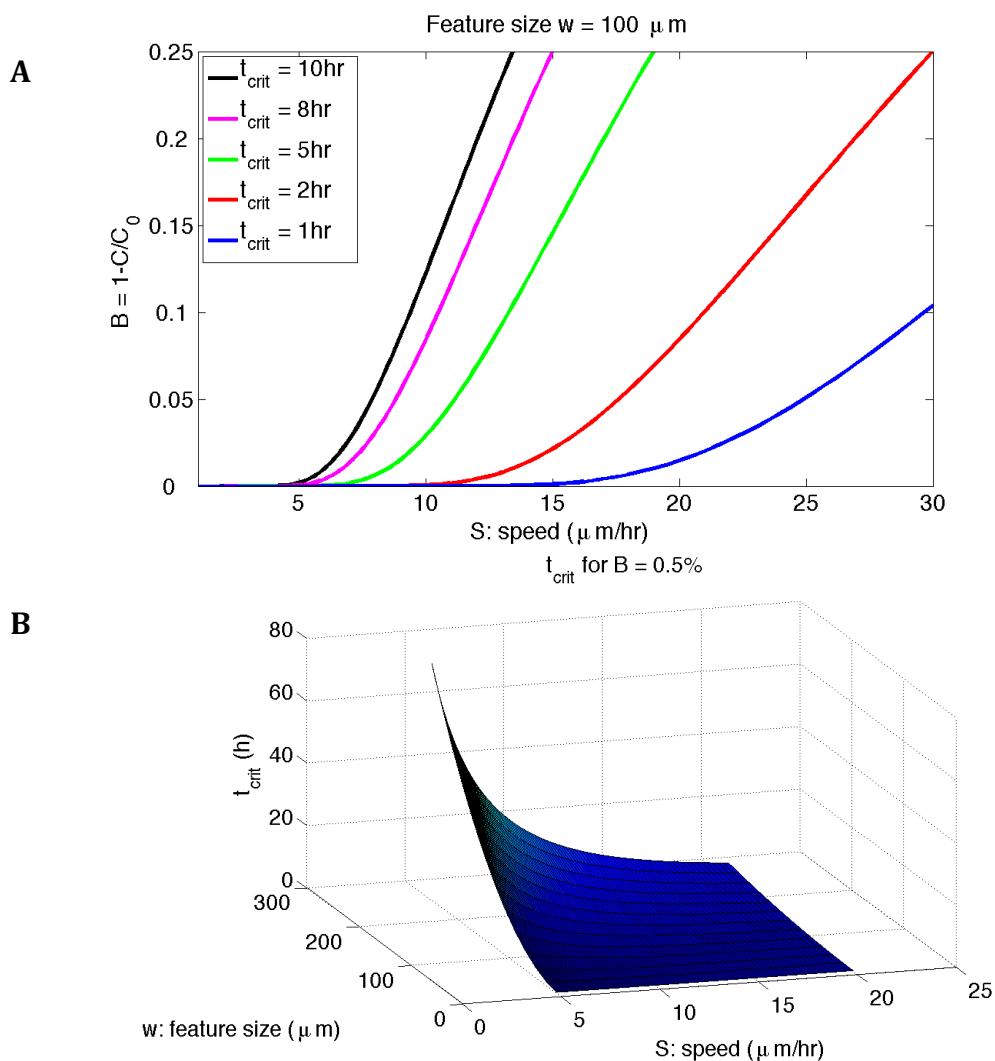
**Figure S10**. *A* – Graph indicating the predicted blurriness in a pattern of 100 μm stripes for different speeds and different values of $t_{crit}$. *B* – Plot showing required $t_{crit}$ value to obtain a blurriness of 0.5% or lower for different pattern sizes and cell speeds.

**Movie SI 1** – co-culture of eGFP and wild type ARPE-19 cells showing cellular re-organization in the cell sheet over a period of 24h. The cells were stained with Hoechst 33342 and imaged every 2 hours for duration of 24 hours.

# Supplemental information: Full description of model

## *Module i*

Module i describes the concentration profile of the inducing agent used to impose the desired pattern of gene expression as a function of time. In this case, module i described a simple stripe of high doxycyline concentration in a background of zero doxycyline, which does not vary over time. Only cells within the stripe are expected to undergo gene induction.

## *Module ii derivation: Defining $t_{crit}$, the time between gene induction and protein expression.*

Module ii defines the time $t_{crit}$ between gene induction and gene product (protein) expression for cells within the stripe described by module i. This defines the time window ($t$=0 to $t$= $t_{crit}$) during which cell migration and hence pattern disruption can occur before the gene expression pattern is switched on. We used a dynamic model to characterize gene expression based on Nevozhay *et al.* [3] and parameter estimates based on fitting flow cytometry experimental data of eGFP (for Tet-on) or mCherry (for Tet-Express) expression levels as a function of time. Specifically the equations of the model were:

$$\frac{d[tetR]}{dt} = \alpha - b \cdot [tetR] \cdot [doxy] - d \cdot [tetR] \qquad EqnS1$$

$$\frac{d[doxy]}{dt} = C - b \cdot [tetR] \cdot [doxy] - f \cdot [doxy] \qquad EqnS2$$

$$\frac{d[GFP]}{dt} = \alpha \cdot \frac{\theta^n}{\theta^n + [tetR]^n} - d \cdot [GFP] \qquad EqnS3$$

Where [tetR], [doxy] and [GFP] represent the concentrations of the free intracellular repressor, inducer and reporter, respectively, according to Nevozhay et al. [3]. For the TetExpress system [GFP] represents mCherry. The parameter C is proportional to the extracellular inducer concentration, the parameter α is the protein synthesis rate, the parameter b is the inducer-repressor association rate, the parameter d is the rate of dilution due to cell growth and the parameter f the combined rate of inducer dilution, outflux and degradation. Cooperative repression is described by the Hill function, where

$\theta$ is the induction threshold and n is the Hill coefficient (fixed at 4).

The output of this ODEs-system is the GFP=$f$(t) profile. We solved this parameter estimation problem using SensSB, a toolbox for systems biology employed through MATLAB [4]. Figures S8A and S8B show the experimental data and corresponding curves fitted using the model for the Tet-on and Tet-Express systems respectively. Tables S2 and 3 show the parameter estimations from the fitted model for the Tet-on system and Tet-Express system respectively.

*Table S2: Parameter estimates for the Tet-on system*

| $\alpha$ (nM d$^{-1}$) | 196.883 | C ([doxy] d$^{-1}$) | 133.4 |
|---|---|---|---|
| b (nM$^{-1}$ d$^{-1}$) | 45.895 | f (d$^{-1}$) | 100.643 |
| d (d$^{-1}$) | 0.778 | $\theta$ (dim-less) | 86.06 |

*Table S3: Parameter estimates for the Tet-Express system*

| $\alpha$ (nM h$^{-1}$) | 45.16 | C ([doxy] h$^{-1}$) | 49.43 |
|---|---|---|---|
| b (nM$^{-1}$ h$^{-1}$) | 9.218 | f (h$^{-1}$) | 2.567 |
| d (h$^{-1}$) | 0.34 | $\theta$ (dim-less) | 6.859 |

Using the model we defined the time $t = t_{crit}$ when [GFP] = 0.9[GFP]$_{maximum}$. For the Tet-on system this was estimated as 155 h and for the Tet-Express system this was estimated at 16.3h.

## *Module iii derivation: Defining the relationship between cell speed and the random walk "diffusion" that leads to pattern dispersion.*

In module iii we relate the speed of cells moving within a sheet to the dispersion of eGFP cells from the stripe pattern during the time window $t$=0 to $t$= $t_{crit}$ defined in module ii. We first estimated the random walk "diffusion" coefficient of eGFP cells within the cell sheet by collecting experimental images of a wide stripe pattern at different times and then quantifying the number of eGFP cells as a function of position and time (Fig. S2). We modelled the random walk motion of eGFP cells migrating within the sheet of non-

labelled cells using the same equations that have previously been used to describe the mixing of two types of densely packed particles at an interface by random walk diffusion. In both cases mixing occurs by random walk motion of one species (cells or particles) into the other species and vice versa at the interface between the two distinct species.

Specifically for the stripe geometry defined by module i, we use an equation that describes random walk diffusion of particles from a stripe with a finite width (Figure 3B). If the region -h < x < h is initially at constant concentration $C_0$ and the region x > h is initially at zero, the equation SI5 given by Edelstein-Keshet, [5] describes dispersion of the particle of interest (in our case eGFP cells) from the pattern over time by random walk motion.

$$C(x,t) = \frac{C_0}{2} \cdot \left[ erf\left(\frac{h-x}{2\sqrt{Dt}}\right) + erf\left(\frac{h+x}{2\sqrt{Dt}}\right) \right] \qquad EqnSI4$$

The stripe source solution can be used, with the width (or feature size) of the initial stripe being $w = 2h$. We set $w = 10000$ μm since this was the geometry of our experimental system and fitted the experimental C(x,t) data using MATLAB's curve fitting toolbox 'cftool ' to estimate D and $C_0$. The results are shown in Figure S2B and the parameter estimates and the 95% confidence intervals are shown in Table SI4 ($R^2 = 0:995$). By defining these parameters D and $C_0$ we could now describe how the concentration of eGFP cells varies as a function of x and t.

**Table SI4: Fit of eGFP cell number C(x,t) data to random walk equation SI4**

| | | |
|---|---|---|
| D | 302.7 (μm²/hr) | Confidence interval: 287.7 – 317.7 |
| $C_0$ | 82.4 (cells/μm) | Confidence interval: 80.7 - 84 |

Previously the "diffusion" of cells was related to the speed of cell migration S, and the cellular persistence time P ((how frequently the cell changes direction) by equation SI5:

$D = \frac{1}{2} S^2 P$     Eqn SI5 [5]

Using equation SI5, our fitted value for D (302.7 $\mu m^2$/hr) and a measured value for S (21 $\mu m$/h) we estimated the value for P to be 82.4 mins. This was in agreement with our experimental measurement of P, which was measured from live cell movies and was 84.8 mins. Confident in the relationship between P, S and D we substituted ½ $S^2P$ for D in our expression to describe the concentration of eGFP cells as a function of x and t. We could now describe the dispersion of eGFP cells over time and space as a function of cell speed.

### Combining modules i, ii and iii: Describing pattern disruption at t = $t_{crit}$

By setting t = $t_{crit}$ (defined from module ii) in Eqn SI4 (defined by module iii) we quantified the dispersion of eGFP cells from the center of the stripe (x=0) over at t=$t_{crit}$. Specifically for width of the stripe w =2h, we solved:

$$\frac{C\left(x = 0, t = t_{crit}\right)}{C_0} = erf\left(\frac{w}{S\sqrt{8Pt_{crit}}}\right) \quad \text{Eqn SI6}$$

for different pattern widths and cell speeds. We then defined the "blurring" of the pattern, **B** that had occurred during the time window as:

$$B = 1 - \frac{C}{C_0} = 1 - erf\left(\frac{w}{S\times\sqrt{8Pt_{crit}}}\right) \quad \text{Eqn SI7}$$

Conceptually B describes the fraction of cells lost from the centre of the original stripe pattern (at x= 0) by random walk movement during the time period $t$=0 to $t$=$t_{crit}$. Graphs shown in the main text show the level of blurriness B expected for different combinations of **S** and **w** and for different values of $t_{crit}$ defined by module ii.

**Materials and Methods**

*Quantification of cell proliferation*.

ARPE-19 cells were seeded on transwells used for patterning gene expression using Tet-On system (see main text materials and methods) either at confluence or sparce. The cells were then fixed with 4% PFA and stained with anti-Ki67 antibody (1:300, Milllipore) and DAPI. The cells were analysed on Olympus IX81 microscope.

*Patterning drug delivery to a confluent sheet of cells*.

To test the ability of parafilm to block penetration of a small molecule drug, $2 \times 105$ cells were seeded on the top surface of the transwell and cultured until full confluency. Next, complete growth medium containing 10 μM CFDA-SE (Invitrogen, Canada) was prepared and placed in the bottom chamber of the transwell system. The cells were incubated in the presence of CFDA-SE for 20 minutes, after which the medium was exchanged to one free of CFDA-SE and cells were incubated for additional 20 minutes. Next, the parafilm membrane was removed, using tweezers, the cells were fixed in 4% PFA paraformaldehyde (Electron Microscopy Sciences, USA), stained with DAPI (Invitrogen, Canada) and analysed on Olympus IX81 microscope.

*Transfer of doxycycline between neighbouring cells*.

Patterned co-cultures of Tet-On-GFP-ARPE-19 cultured in the presence of doxycycline for one week and Tet-On-GFP-ARPE-19 cells cultured in the absence of doxycycline were prepared using a method that facilitates minimum disruption of patterns over prolonged periods of time, as described previously [2]. The co-cultures were comprised of 1-cm wide stripes of each cell type adjacent to each other. The co-culture samples were then maintained in the absence of doxycycline for 6 days, after which they were fixed in 4% PFA, stained with DAPI and imaged on Olympus IX81 microscope.

## References

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2. S. Javaherian, K. A. O'Donnell, A. P. McGuigan, A fast and accessible methodology for micro-patterning cells on standard culture substrates using Parafilm inserts. *PloS one* 2011, *6*. e20909, DOI: 10.1371/journal.pone.0020909.

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4. M. Rodriguez-Fernandez, J. R. Banga, SensSB: a software toolbox for the development and sensitivity analysis of systems biology models. *Bioinformatics* 2010, *26*. 1675-6, DOI: 10.1093/bioinformatics/btq242.

5. L. Edelstein-Keshet, *Mathematical Models in Biology*. McGraw-Hill Companies: 1987.