# The Precision Control of Autophagic Flux and Vesicle Dynamics—A Micropattern Approach

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**Supplementary Materials:**

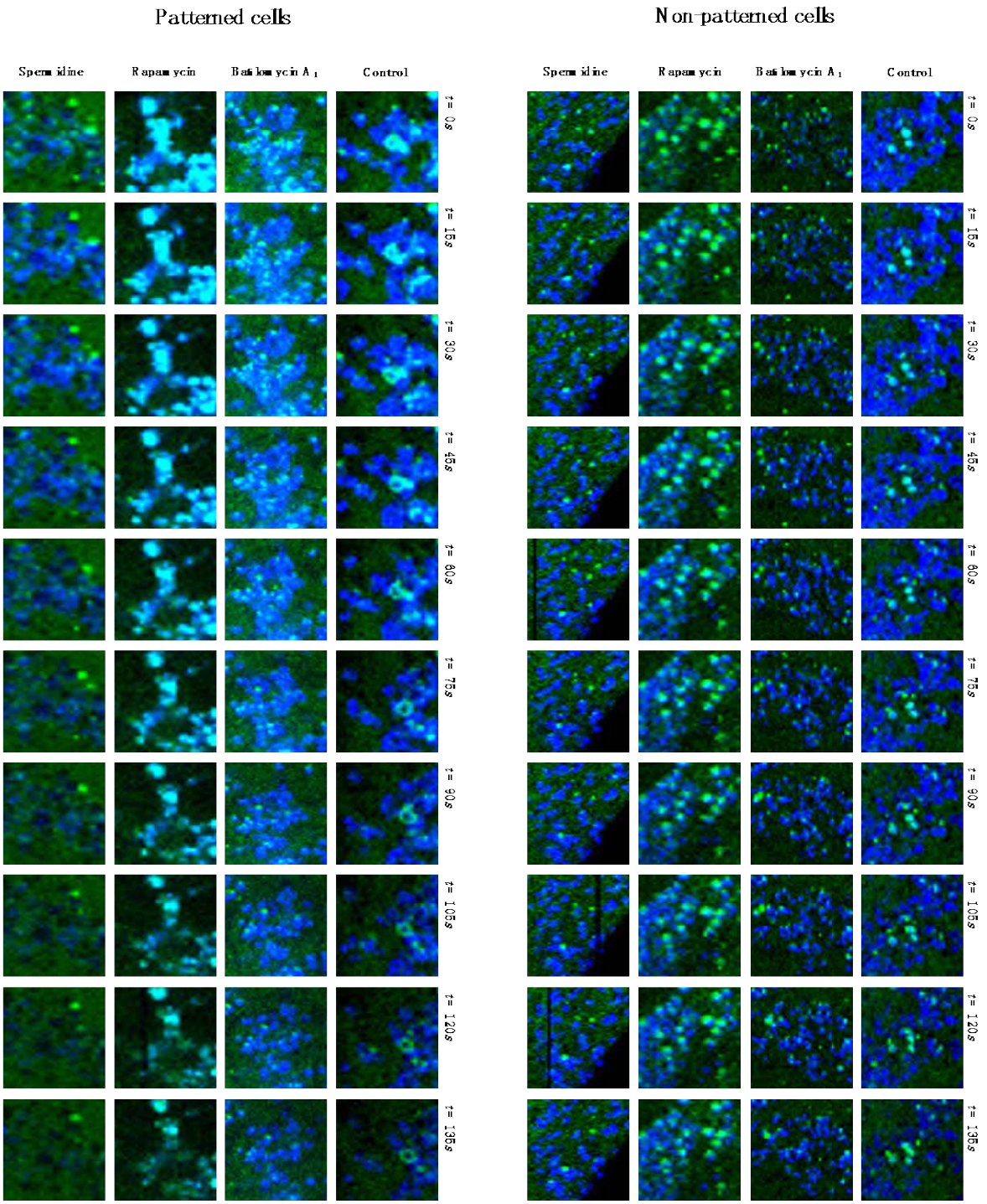


**Figure S1.** Time lapse micrographs indicating distinct pool size distribution in patterned and non-patterned cells upon autophagy induction, using rapamycin or spermidine, as well as partial inhibition, using non-saturating concentrations of bafilomycin.

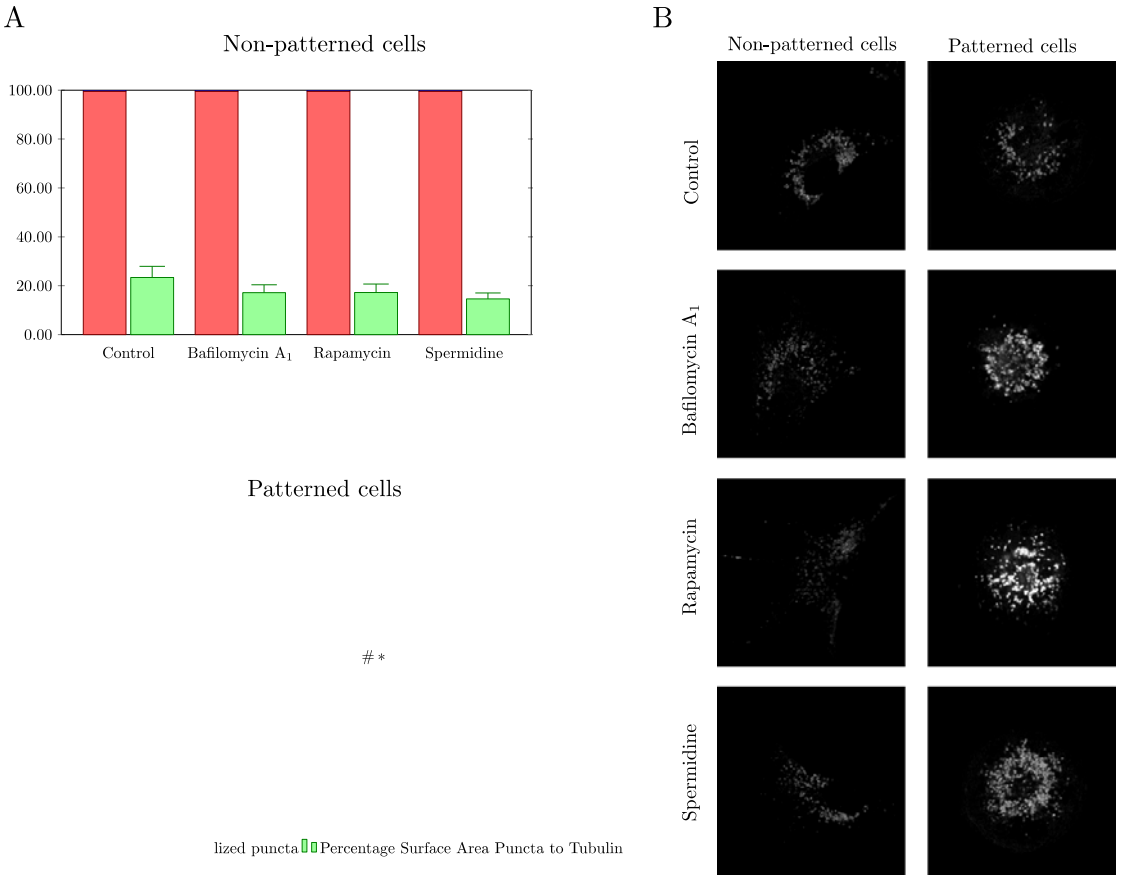


**Figure S2.** Colocalization analysis (a) indicating colocalization of autophagosomes with the microtubule network. Micrographs indicating colocalization profile in patterned and non-patterned cells (b) upon autophagy induction, using rapamycin or spermidine, as well as partial inhibition, using non-saturating concentrations of bafilomycin.

**Data processing**

Data were read into and processed using combinations of standard Mathematica® functions and graphical tools.

**Tracking of particles over a number of time-steps**

The figures below show the histogram of the number of puncta that can be tracked up to precisely $\tau$ time-steps from the initial time.

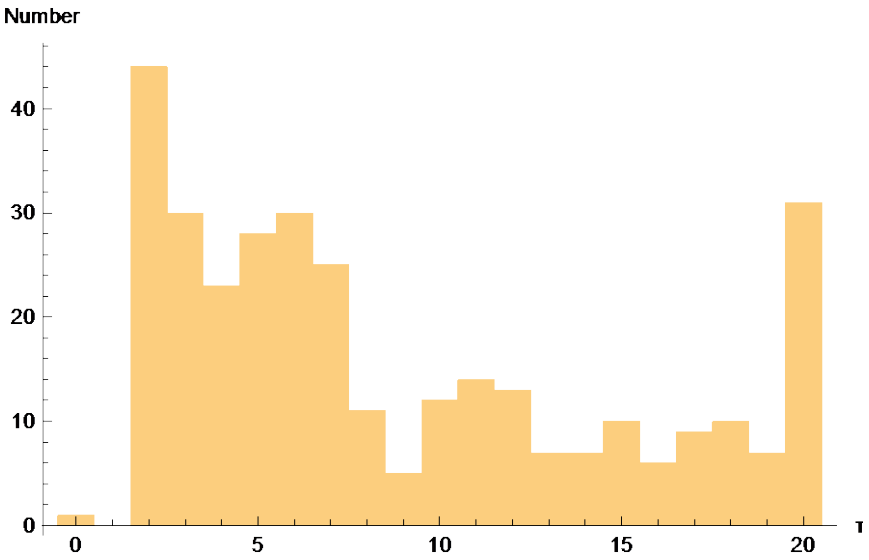


**Figure S3 (a):** Histogram for number of particles trackable up to exactly time step $\tau$. Dataset micropatterned cells.

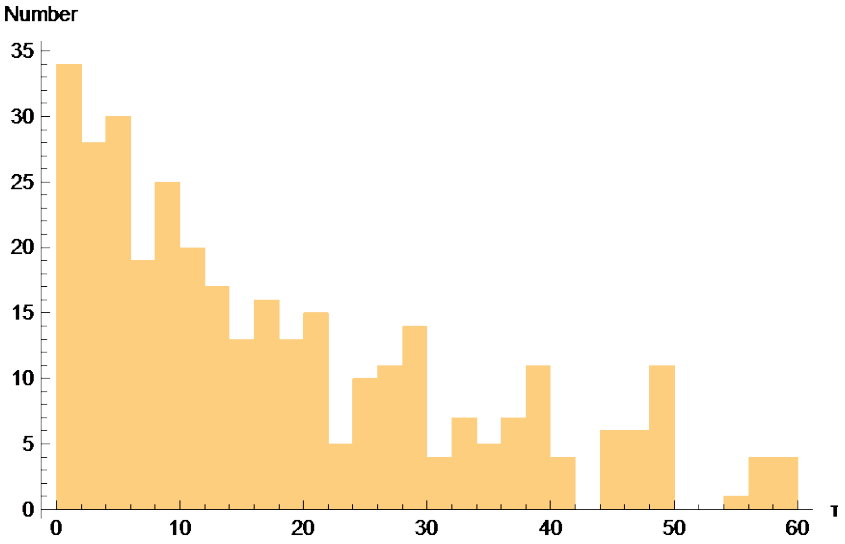


**Figure S3 (b):** Histogram for number of particles trackable up to exactly time step $\tau$. Dataset non-patterned cells.

**Puncta end-to-end distance distribution throughout the cells**

For an ideal random walk over $\tau$ time steps, the average of the square of end-to-end distance is proportional to $\tau$, or,

$$\overline{R^2} \sim \tau.$$

On average the puncta produce the same scaling behaviour as an ideal random walk, cf. Figure S4. However, we note that the other results of current and order with respect to a radial vector show that, in spite of a reasonable match to the ideal random walk scaling, the puncta do not behave as random walks.

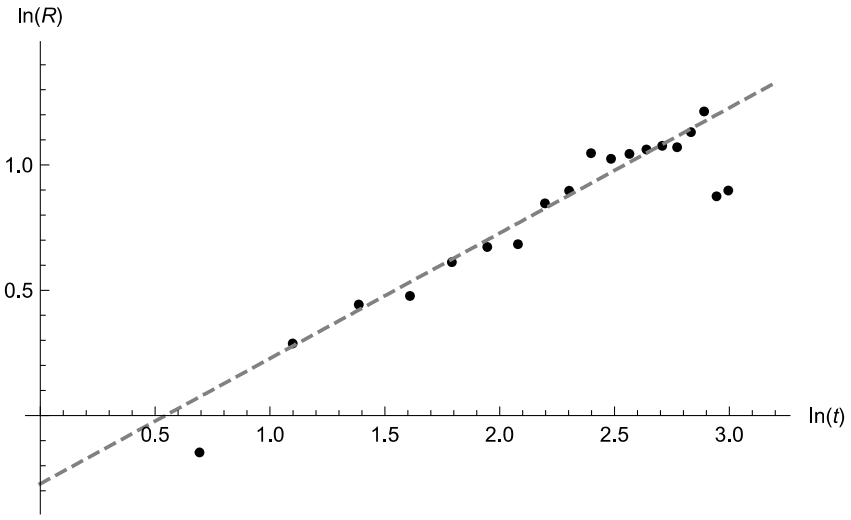


**Figure S4 (a):** Plot of $\ln\sqrt{\overline{R^2}}$ vs $\ln\tau$ for micropatterned cells, with the dashed line to indicate the ideal random walk scaling. Note, that the presence of such scaling is not sufficient evidence for a random walk.

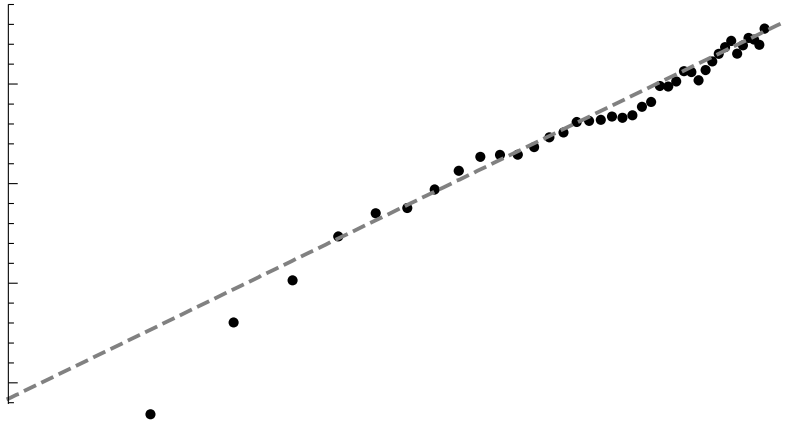

**Figure S4 (b):** Plot of $\ln\sqrt{\overline{R^2}}$ vs $\ln\tau$ for micropatterned cells, with the dashed line to indicate the ideal random walk scaling. Note, that the presence of such scaling is not sufficient evidence for a random walk.

**Determining $Q_\tau$**

The quantity $Q_\tau$ is crucial in determining the order over more than one time-step:

$$Q_\tau = \frac{1}{N(S)} \sum_{n \in S} \left(1 - 2\left(\hat{r}_{n,\tau} \cdot \hat{v}_{n,\tau}\right)^2\right).$$

Here the quantity $\hat{r}_{n,\tau}$ is the unit vector determined from the average position of the punctum $n$ for time-steps 1 to $\tau$. The unit vector for the velocity $\hat{v}_{n,\tau}$ is determined by taking the difference of the

vector position of the punctum at time-step $\tau$ and time-step 1, and by diving by the number of time steps.

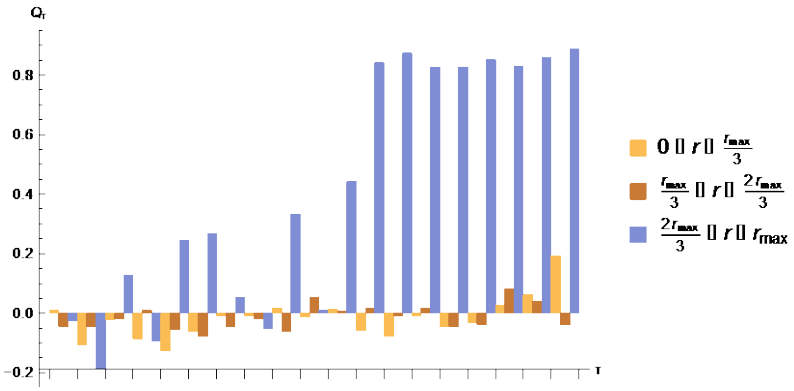


**Figure S5 (a):** Order measure for puncta of dataset micropatterned cells, where data are collected over tracks spanning at least $\tau$ time-steps. For tracks of longer duration strong order is noticeable in the region near the periphery. Here the maximum distance is taken as 30 units.

For non-patterned cells, we find similar behaviour of this quantity, as in the micropatterned cells described in the main text.

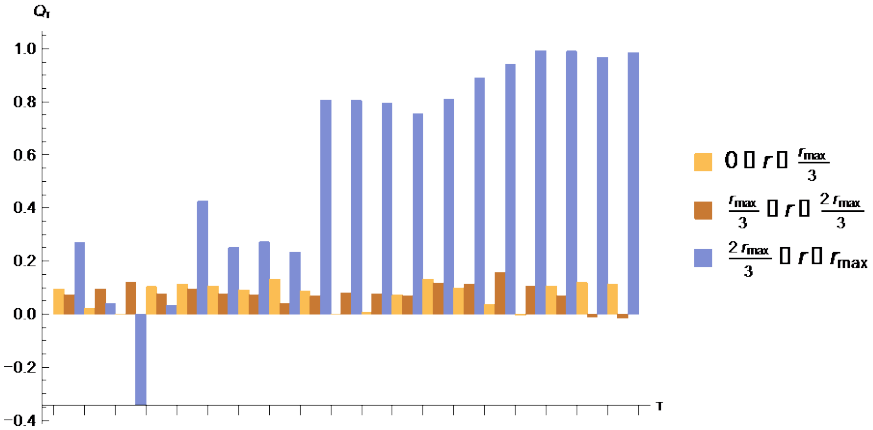


**Figure S5 (b):** Order measure for puncta of dataset non-patterned cells, where data are collected over tracks spanning at least $\tau$ time-steps. For tracks of longer duration strong order is noticeable in the region near the periphery. Here the maximum distance is taken as 60 units.