**Supplementary Figures**

**Transiently heritable fates and quorum sensing drive early IFN-I response dynamics**

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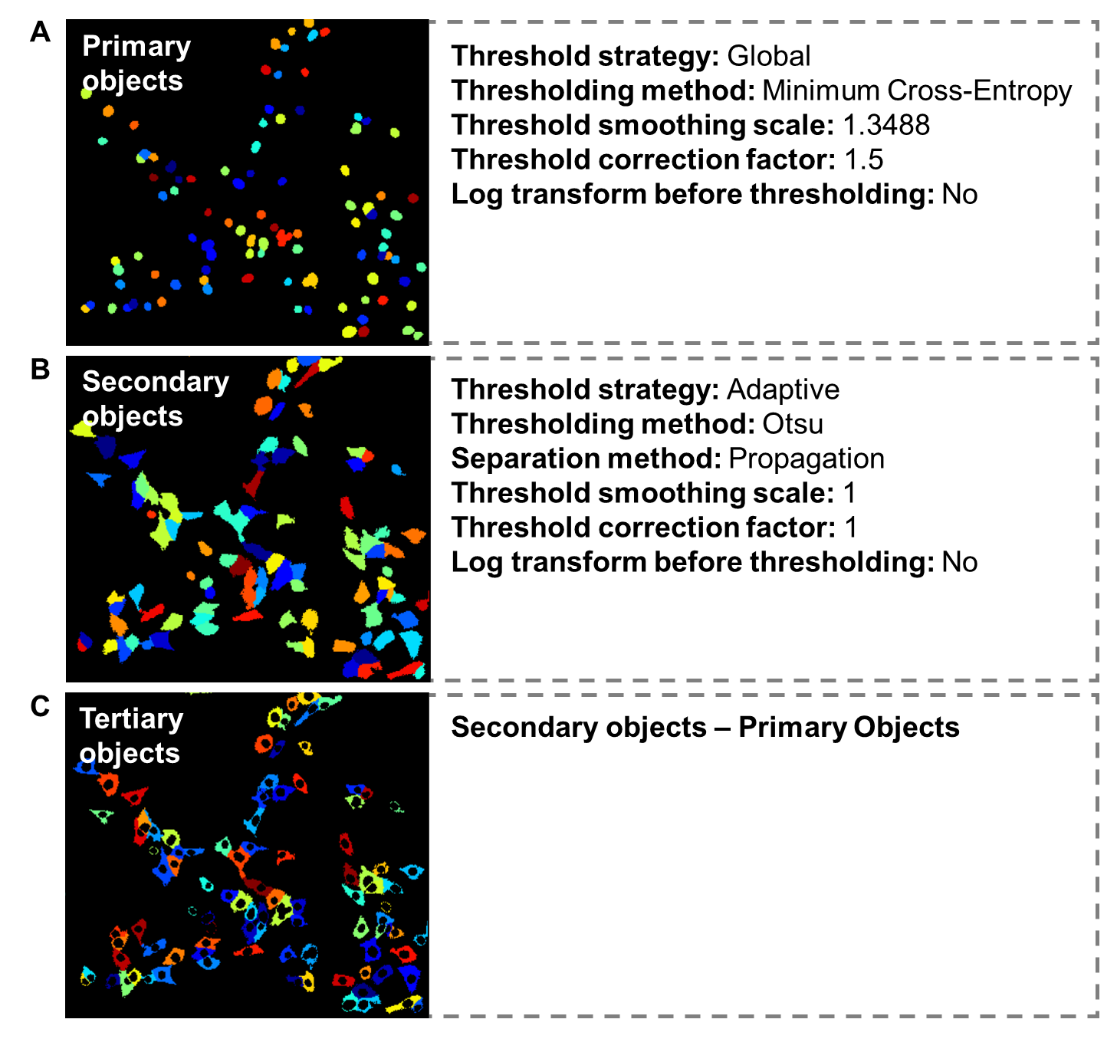
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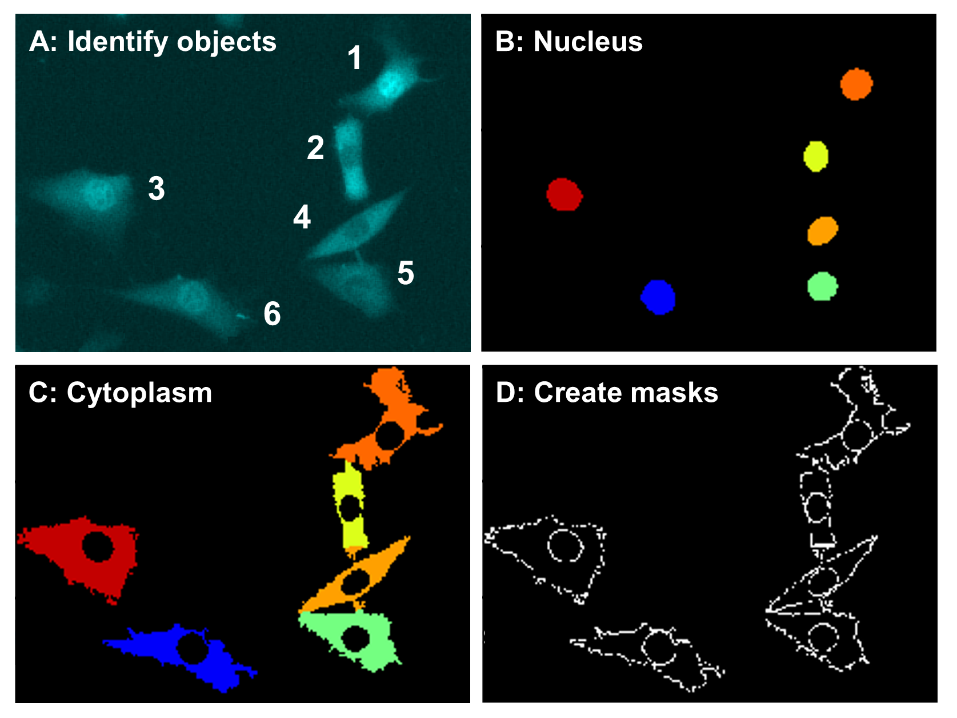
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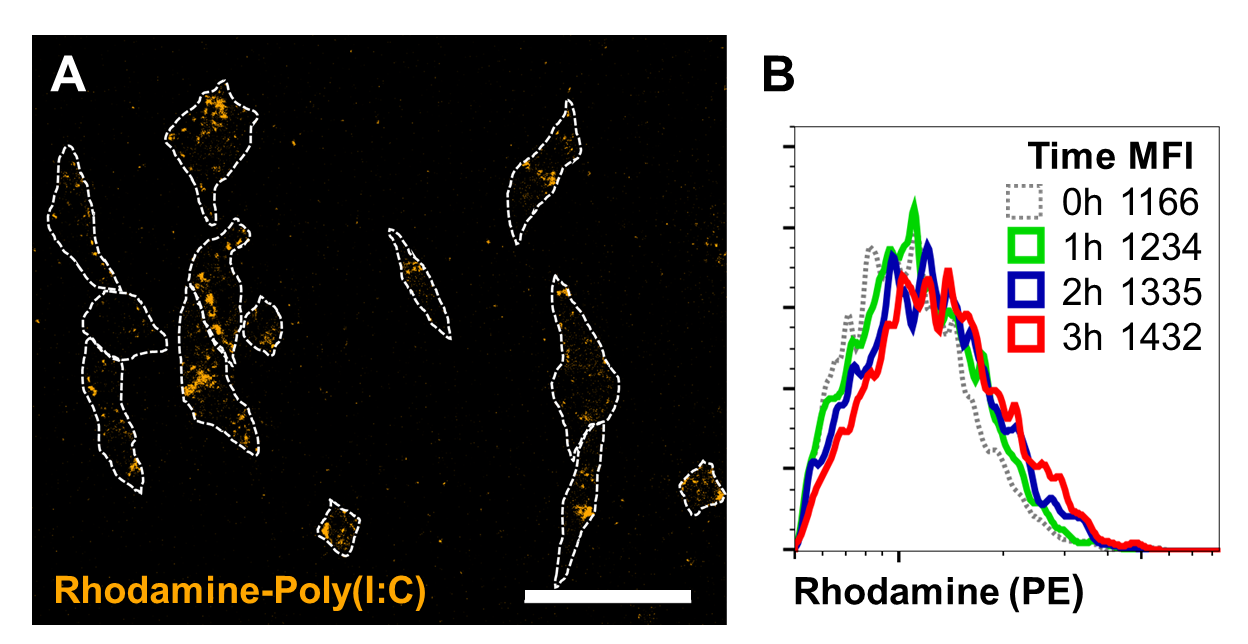
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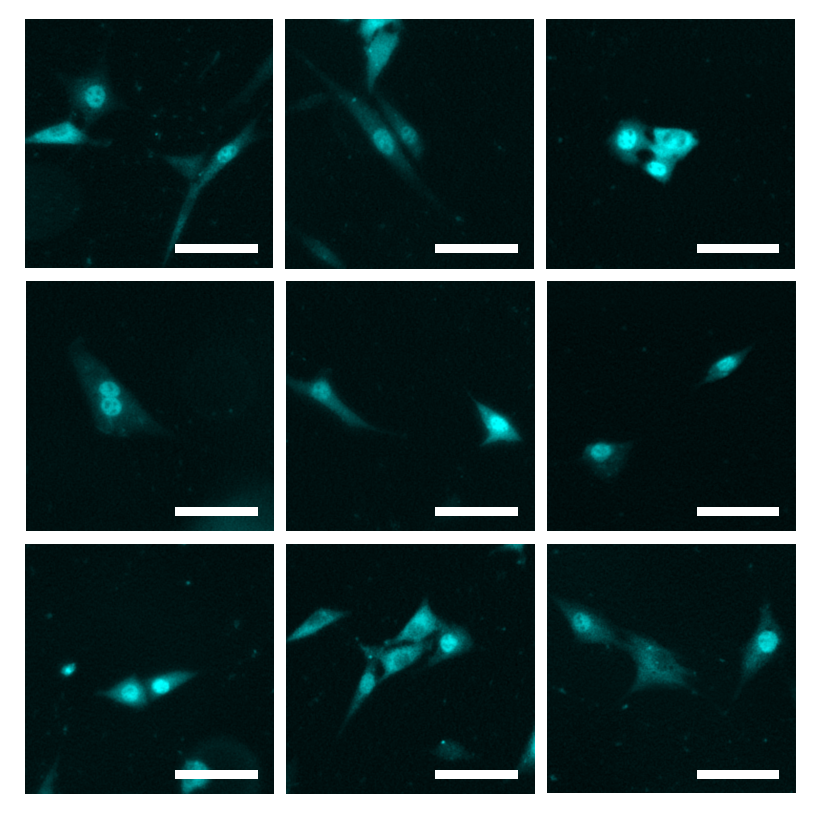
**Supplementary Figure 1.** Details on automated script in CellProfiler software.(**A-C**)Identification of primary, secondary and tertiary objects.



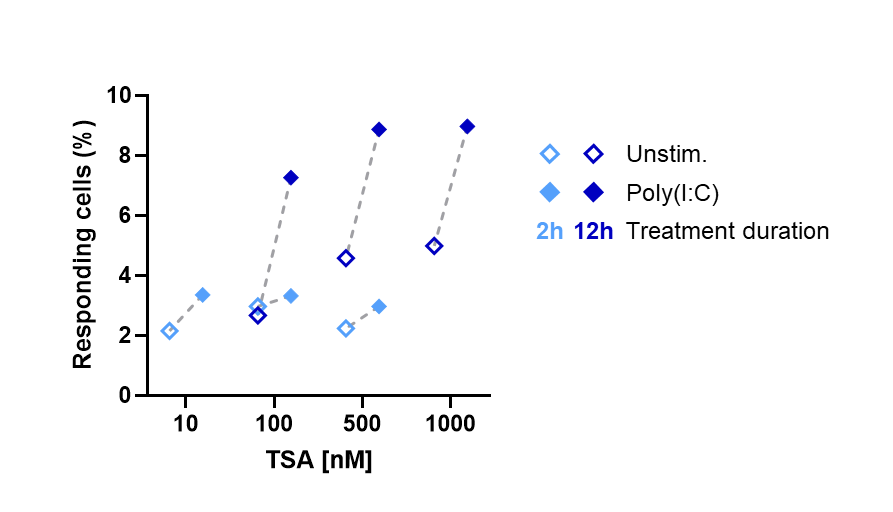
**Supplementary Figure 2.** Detailed automated script analysis performance.(**A**)Identification and indexing of objects**.** (**B**) Detection of nuclei. (**C**) Detection of cytoplasm. (**D**) Creation of masks aligning the nuclei and cytoplasm.



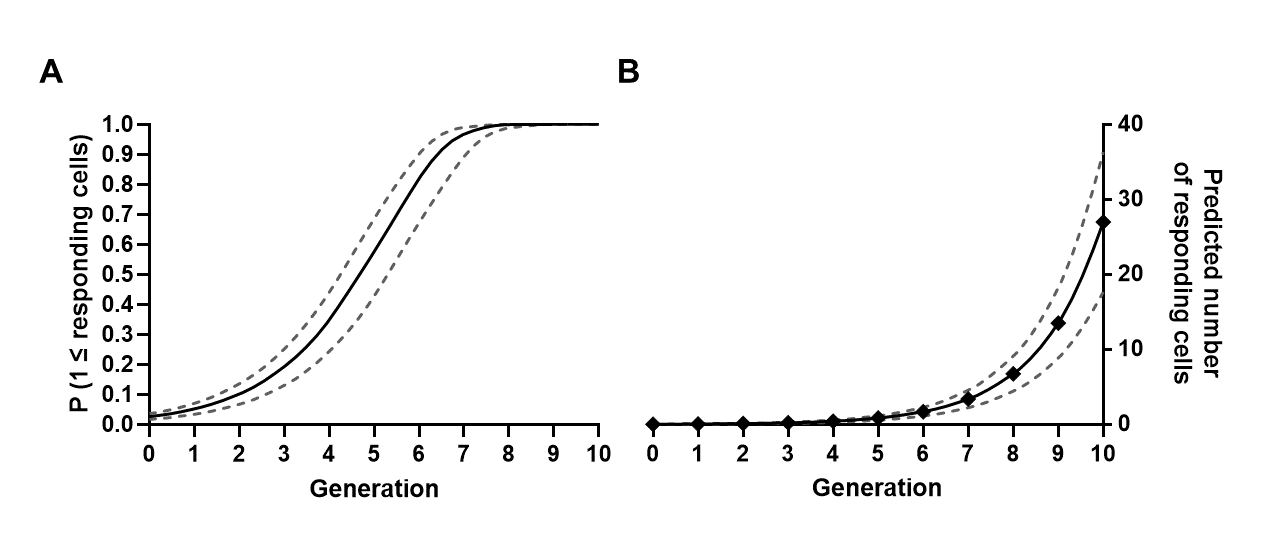
**Supplementary Figure 3.** Confocal microscopyand flowcytometry analysis of transfection efficiency. (**A**)Fibroblasts were transfected as described previously. After 3 hours, cells were washed, fixed, and analyzed using confocal microscopy. Scale bar equals 100 μm. (**B**) Fibroblasts were transfected, incubated, trypsinized, thoroughly washed, and measured using flow cytometry. Depicted are the total fibroblast events, for unstimulated (0h), and for the first 3 hours after transfection, and their corresponding fluorescent mean fluorescent intensity (MFI) values for PE-Rhodamine.



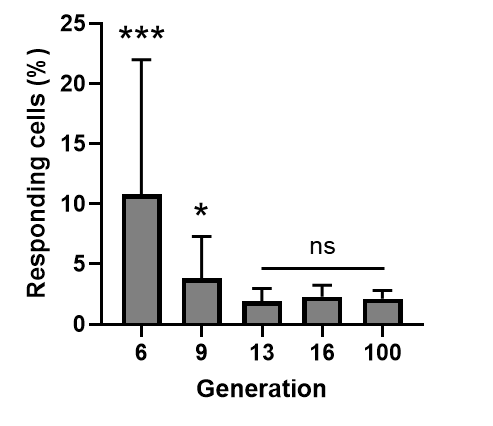
**Supplementary Figure 4.** Microscopy images of numerous neighboring cells showing translocation.Several examples of neighboring cells showing translocation, transfected with 2.5 μg/mL Poly(I:C) for 7 hours, imaged and analyzed for IRF7 translocation. +20% Brightness and +20% contrast were applied for visualization purposes. Scale bar, 100 μm.



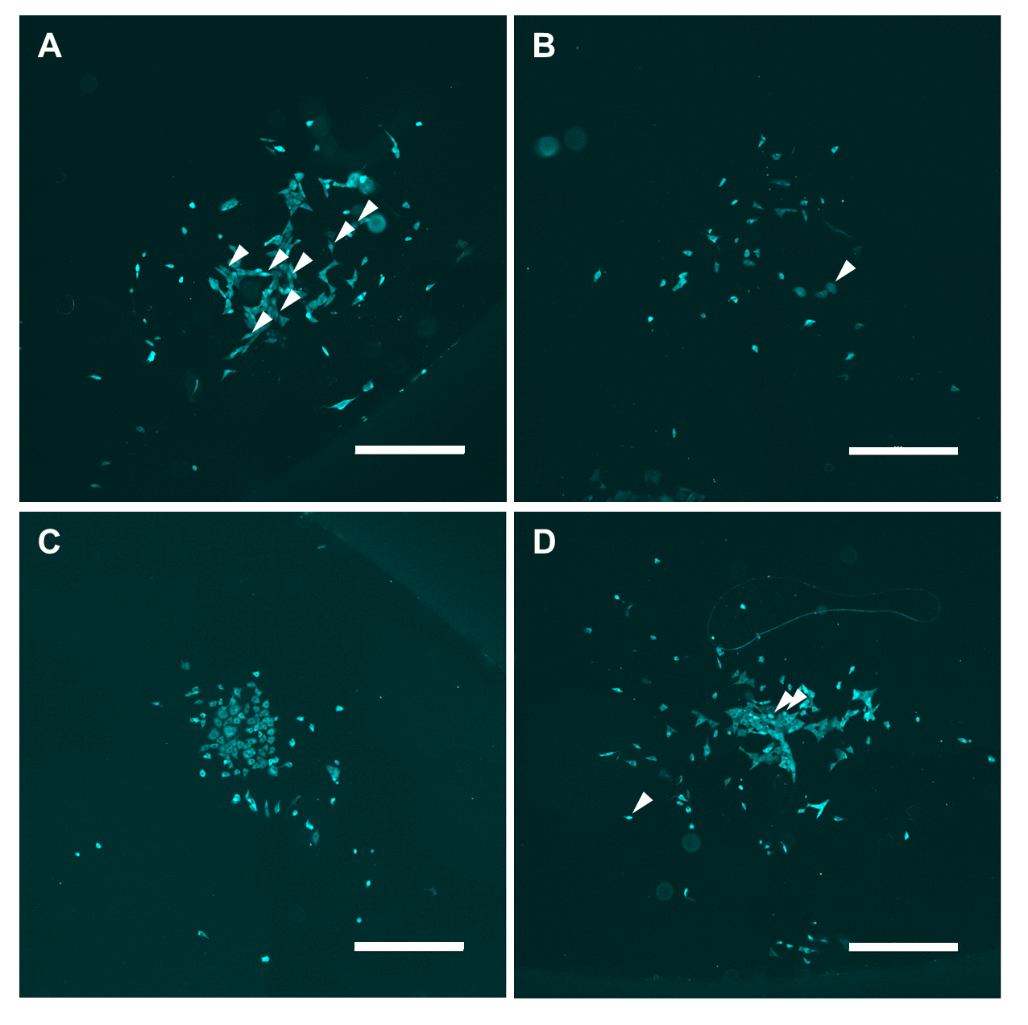
**Supplementary Figure 5.** Percentages of responding cells upon treatment with varying concentration of HDACi TSA, for varying durations.



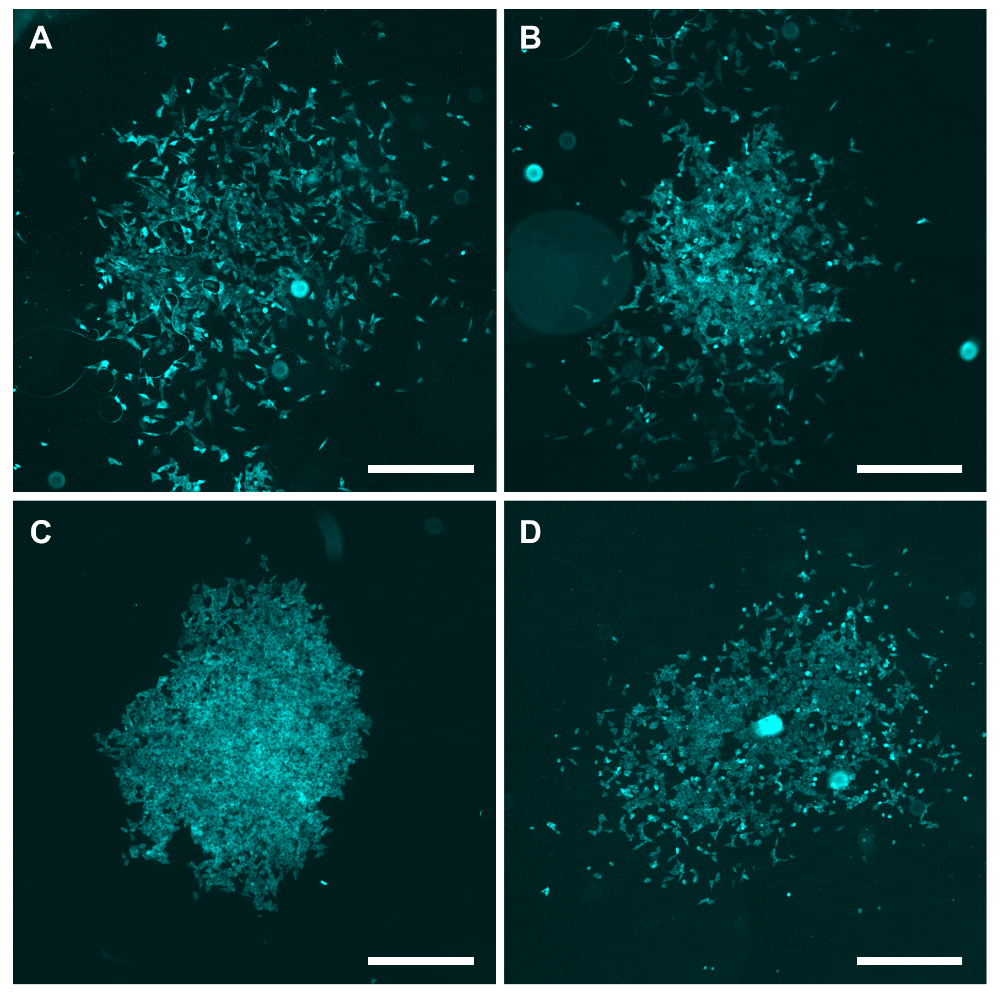
**Supplementary Figure 6.** Probability calculations.(**A**) Probability curves for the presence of at least one first responder per clone over the first 10 generations, assuming stochasticity. Solid line represents the probability based on the mean percentage of first responders in regular cultures; dashed lines represent the mean plus and minus the standard deviation (SD). (**B**) Curves on predicted numbers of first responders present over the first 10 generations, based on the mean percentages obtained from regular cultures (solid line), and the mean +/- SD (dashed line).



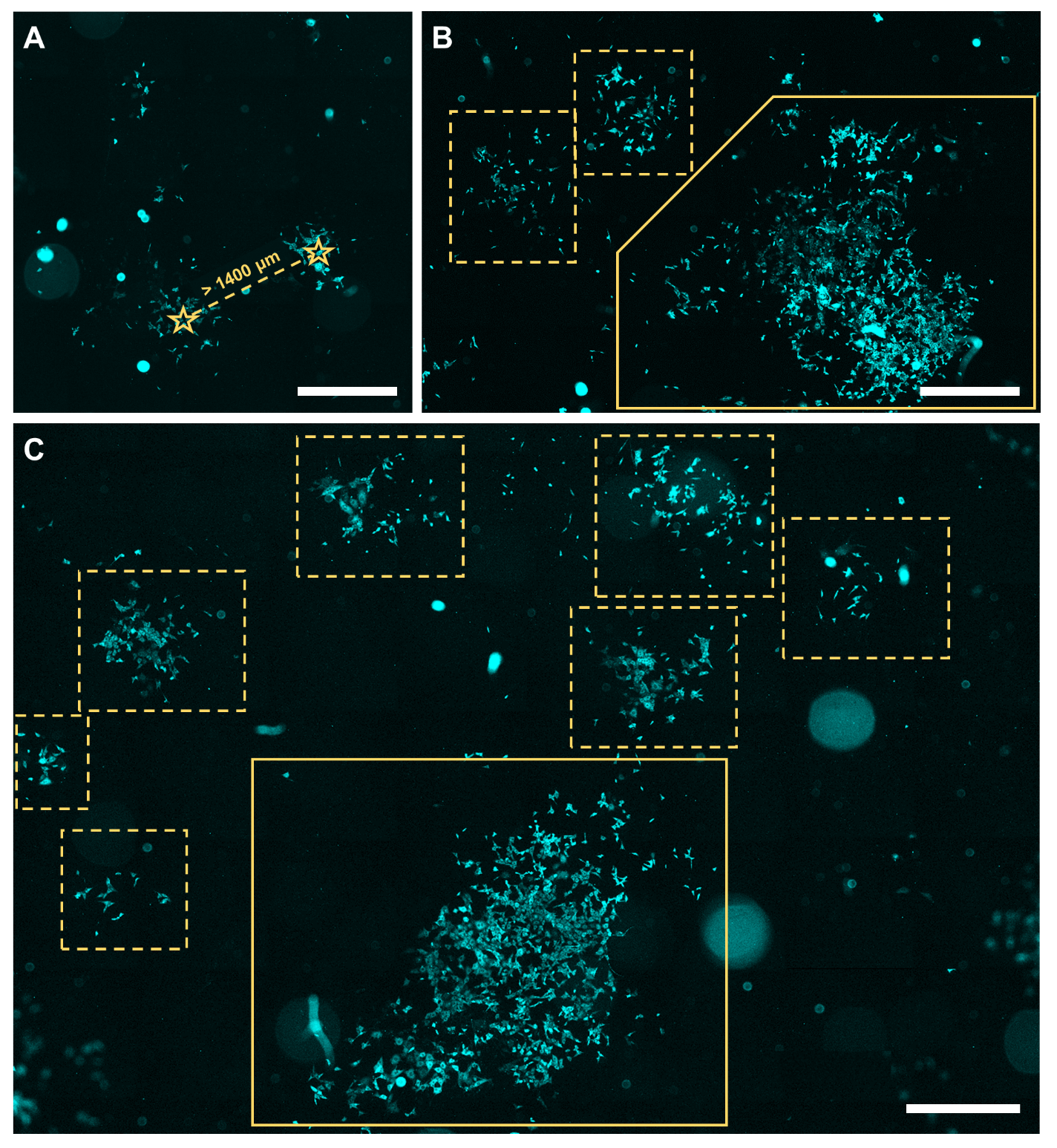
**Supplementary Figure 7.** Statistical analysis on fluctuation data. Percentages of responding cells of clones of different generations. Data are represented as mean ± SD; Welch’s t test, two-tailed; \*\*\*p < 0.001 ; \*p = 0.0446.



**Supplementary Figure 8.** Microscopy images of clones of generation 6. (**A-D**)Several examples of clones of generation 6, transfected with 2.5 μg/mL Poly(I:C) for 7 hours, imaged and analyzed for IRF7 translocation, displaying numerous translocated cells, some of which are indicated with white arrows. +20% Brightness and +20% contrast were applied for visualization purposes. Scale bar equals 500 μm.



**Supplementary Figure 9.** Microscopy images of clones of generation 9.(**A-D**) Several examples of clones of generation 9, transfected with 2.5 μg/mL Poly(I:C) for 7 hours, imaged and analyzed for IRF7 translocation. +20% Brightness and +20% contrast were applied for visualization purposes. Scale bar equals 500 μm.



**Supplementary Figure 10.** Microscopy images of cells of generation 6 seeded varying seeding densities. (A) Visualization of two clusters of cells that were considered two separate clones, with over 140 μm distance between the centers of the clusters, depicted by the star symbol. (**B**, **C**) Several examples of clones of generation 6, in dashed boxes, with in the same field a grouped clone, in solid box. +20% Brightness and +20% contrast were applied for visualization purposes. Scale bar equals 1000 μm.