Supplemental material

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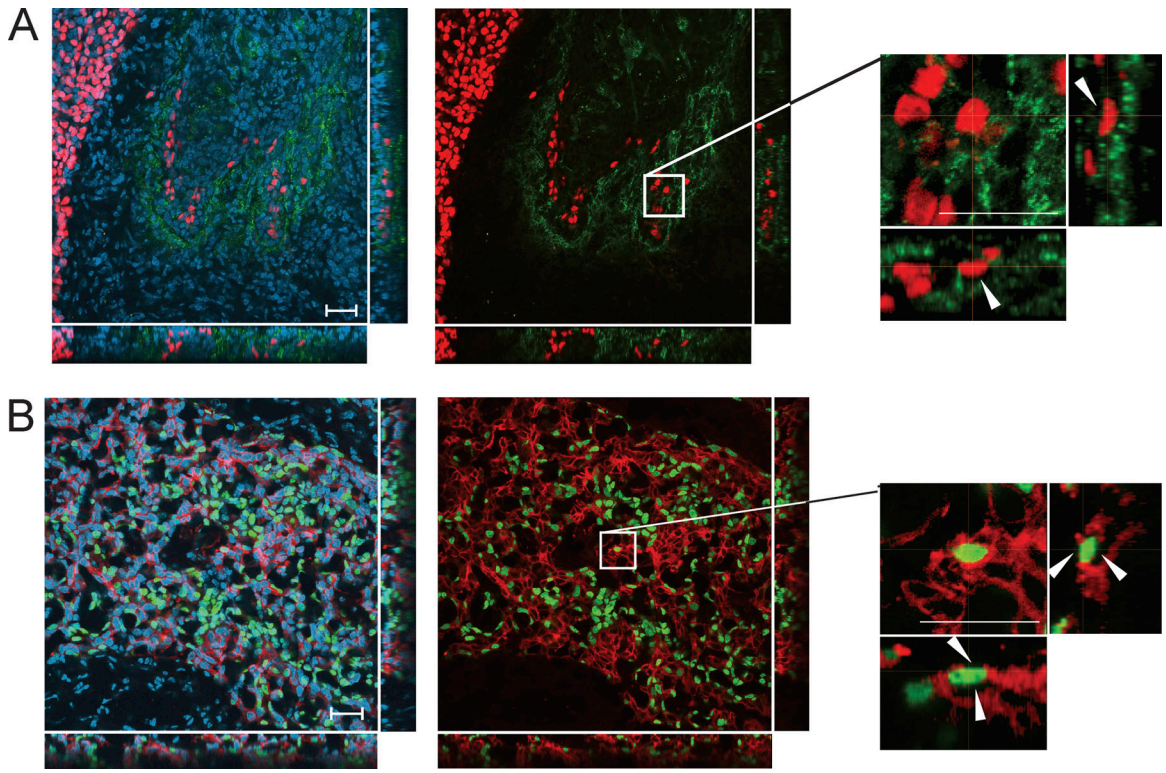


Figure S1. **Examples of images in treated MMTV-*Wnt1* tumors that are not considered to be cell-in-cell structures.** Additional images from experiments presented in Fig. 1 (A and B). **(A)** Tumor cells expressing either H2B mCherry or cytoplasmic GFP were mixed, transplanted, treated, and imaged. Z-stack projections display 20 images taken in 2-µm steps, with a total z-range of 37.82 µm. **(B)** Tumor cells expressing either H2B GFP or farnesylated mCherry were mixed, transplanted, treated, and imaged. Z-stack projections display 24 images taken in 2-µm steps, with a total z-range of 45.88 µm. Arrowheads indicate incomplete or inconclusive engulfment. Scale bars represent 50 µm; 25 µm in zoomed images.

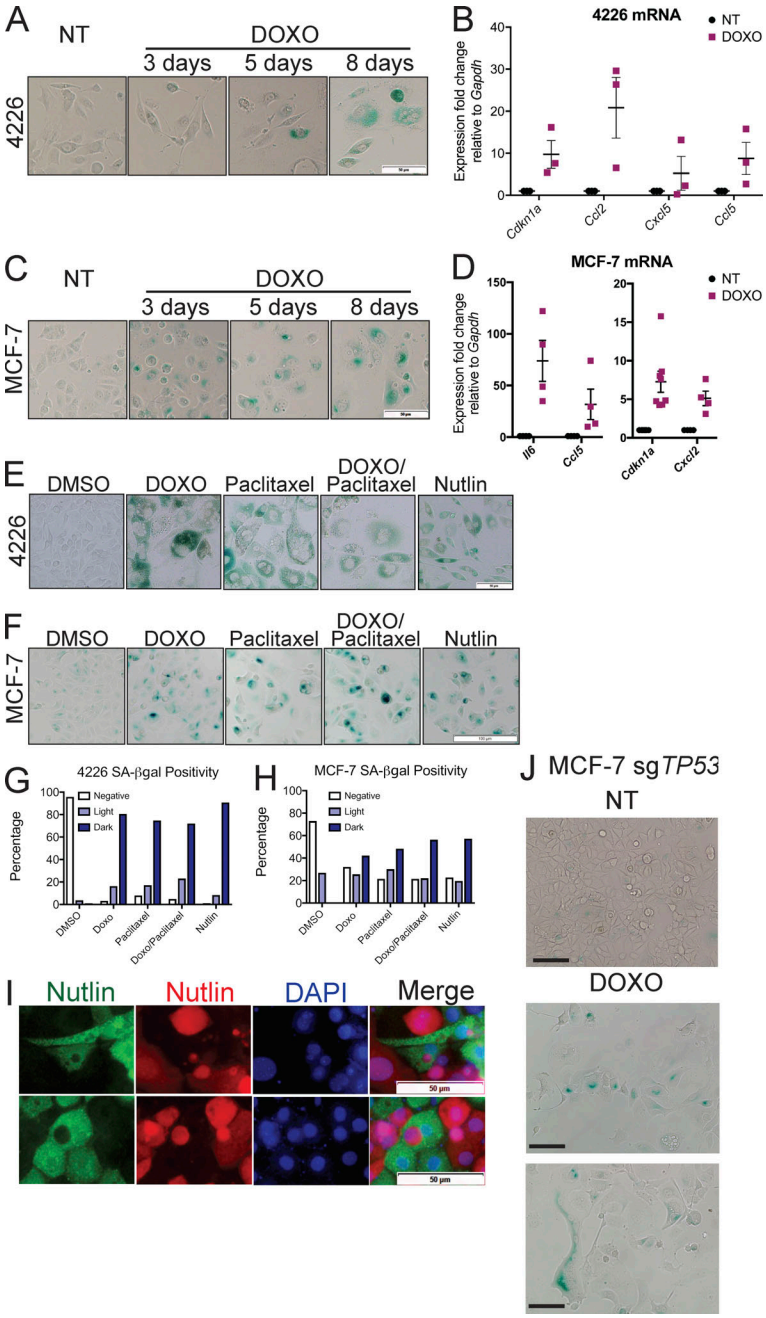


Figure S2. **Senescent phenotypes are expressed by chemotherapy-treated breast cancer cell lines. (A)** Staining for SA-βGal activity was performed on senescent doxorubicin (DOXO)-treated 4226 cells in four separate experiments. In each experiment, at least five random fields of view were taken. **(B)** RNA was isolated from 4226 cells from three independent experiments. Quantitative RT-PCR was performed in duplicate. Levels of expression relative to *Gapdh* were determined using the method of Pfaffl (2001). Although the trend shows an increase in expression of each gene, the error bars were too large to be deemed significant by *t* test. **(C)** Staining for SA-βGal activity was performed on senescent doxorubicin-treated MCF-7 cells in three separate experiments. In each experiment, at least five random fields of view were taken. **(D)** RNA was isolated from MCF-7 cells from four independent experiments. Quantitative RT-PCR was performed in duplicate. Relative levels of expression relative to *Gapdh* were determined. Although the trend shows an increase in expression of *Ccl5*, the error bars were too large to be deemed significant by *t* test. *Cdkn1a*, *Cxcl2*, and *Il6* had P values of <0.001, <0.01, and <0.05, respectively. **(E)** 4226 cells were plated and then treated with drugs as indicated: 0.75 µM DOXO, 10 nM Paclitaxel, or both drugs combined, and changed 24 h later. Nutlin was applied every other day to a final concentration of 15 µM. 8 d later, SA-βGal assay was performed. Three separate experiments were performed; at least five images were taken per condition each time. Representative images shown. **(F)** MCF-7 cells were plated and then treated with drugs as indicated: 0.25 µM DOXO, 5 nM Paclitaxel, or both drugs combined, and changed 24 h later. Nutlin was applied every other day to a final concentration of 2.5 µM. 8 d later, SA-βGal assay was performed. Two experiments were performed at least five images were taken per condition; representative images shown. **(G and H)** Quantifications of SA-βGal positivity from representative experiments pictured in E and F. **(I)** 4226 cells expressing GFP or mCherry were mixed and then treated with 15 µM nutlin for 8 d. Representative images shown from experiments discussed in Fig. 2 C. Scale bars represent 50 µm (A, C, E, and I) and 100 µm (F). **(J)** MCF-7 sg*TP53* cells were treated with doxorubicin as in F, and 7 d later the treated cells and NT cells were stained for SA-βGal as in F. Representative images were captured; scale bars represent 50 µm.

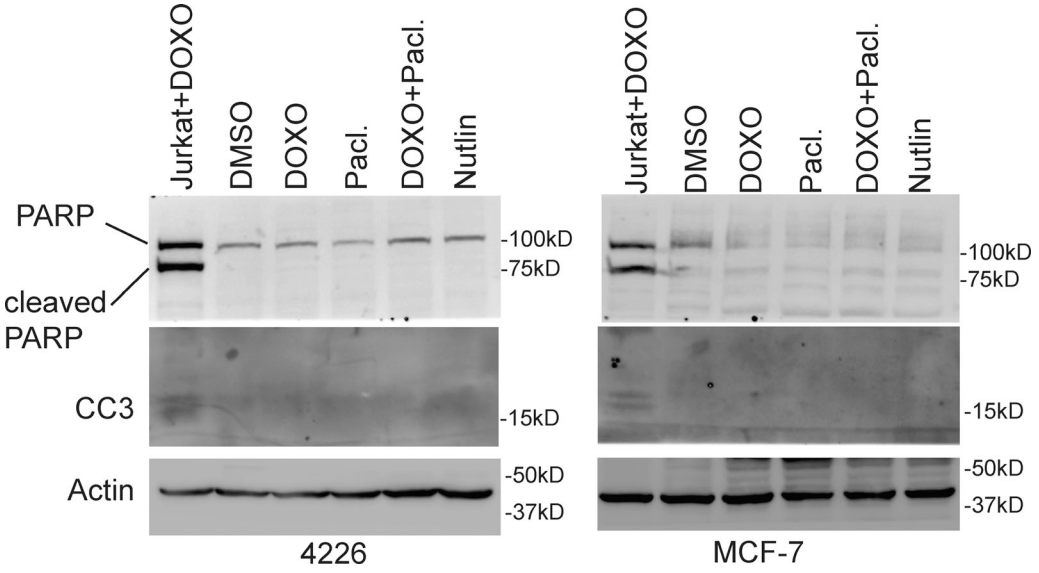


Figure S3. **Expression of apoptotic markers in treated breast cancer cell lines.** Indicated cell lines were treated with DMSO control, doxorubicin (DOXO), paclitaxel (Pacl.), doxorubicin plus paclitaxel, or nutlin as in Fig. 2 (C and D), and then were harvested after 7 d. Immunoblots were performed with antibodies to PARP (detecting the full-length and cleaved isoforms) and cleaved caspase 3, followed by actin. Lysates from Jurkat cells treated for 24 h with 250 nM doxorubicin are included as positive controls. Molecular weight markers are indicated at the right of each gel.

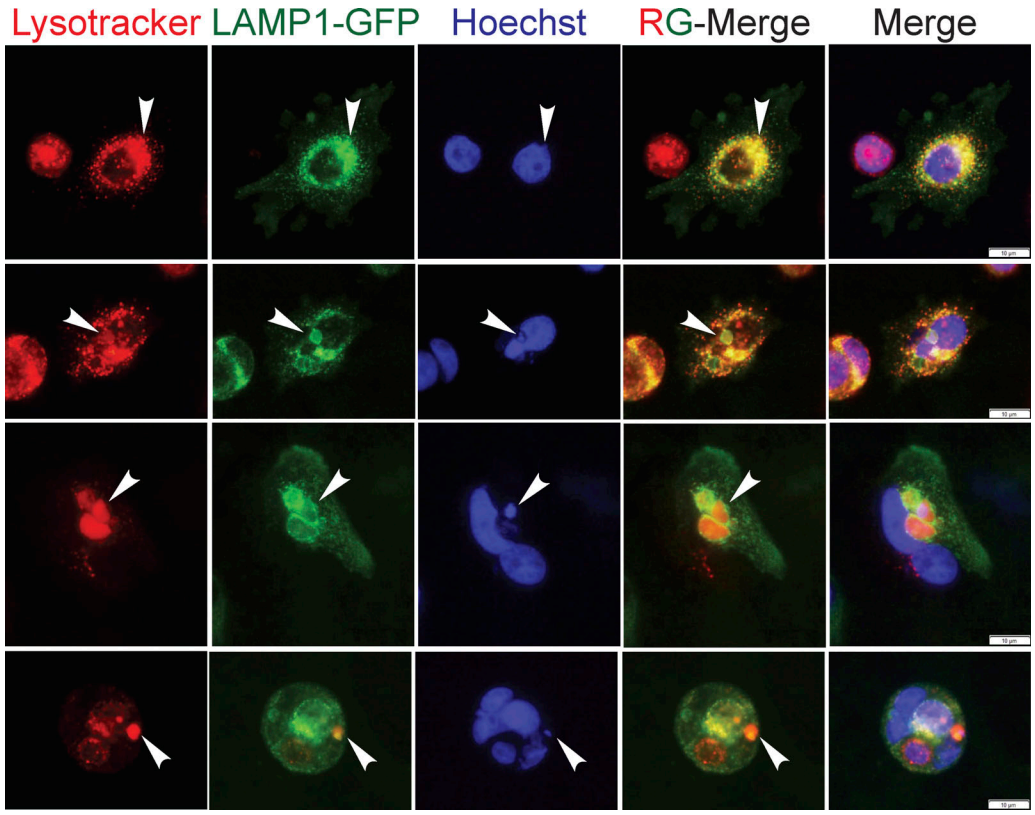


Figure S4. **LysoTracker and LAMP-1 GFP colocalize to engulfed cells within senescent cells.** LAMP1-GFP–expressing MCF-7 cells were treated with 0.25 µM doxorubicin for 24 h and then mixed with NT parental MCF-7 cells. 7 d later, cultures were fixed and stained with LysoTracker, and images were captured of senescent cells with engulfed clear cells (white arrowheads). LAMP1 MCF-7 cells were stained with LysoTracker in one experiment; representative images of engulfed cells are shown. Scale bars represent 10 µm.

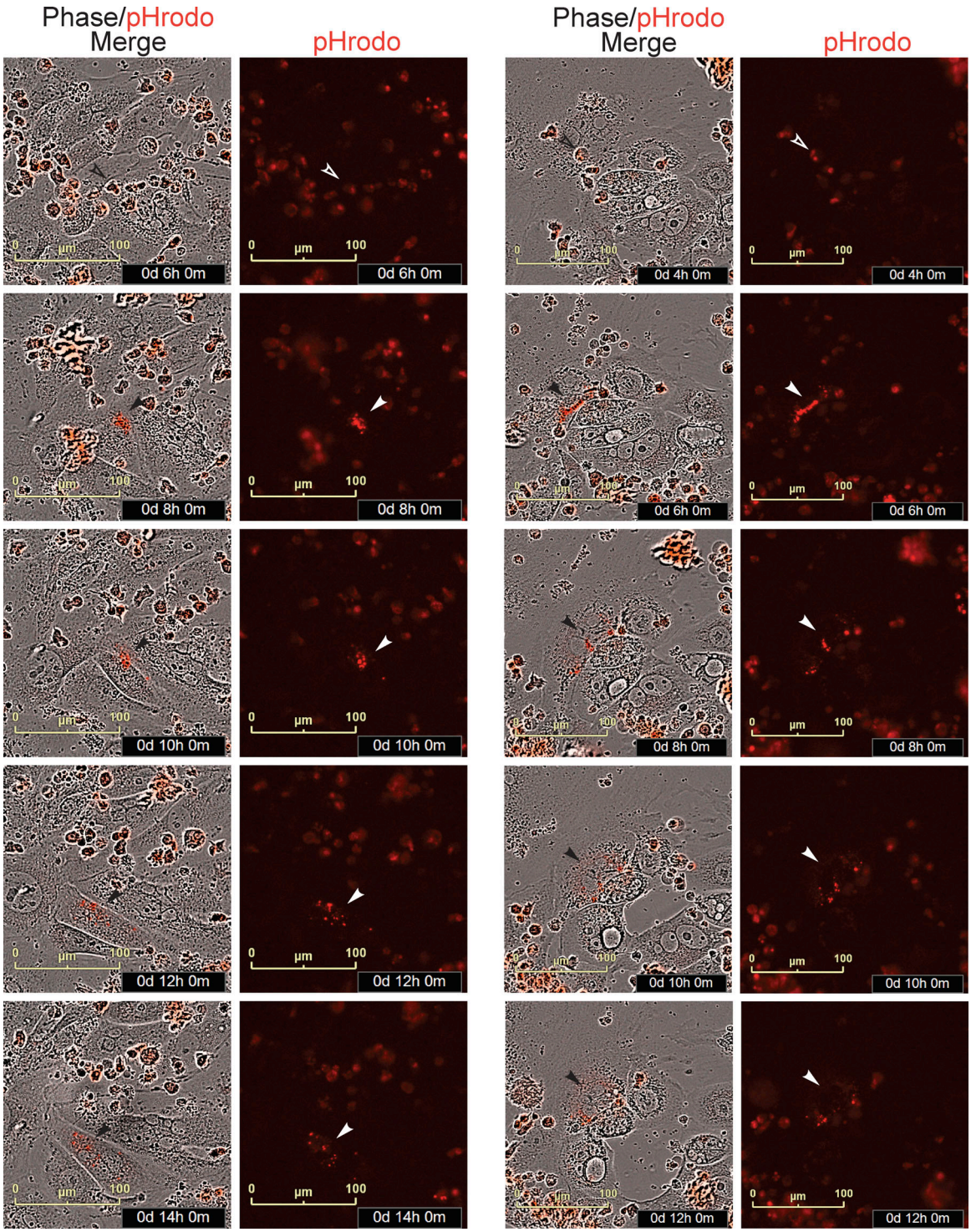
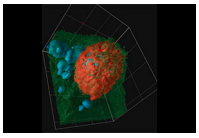
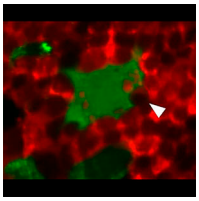


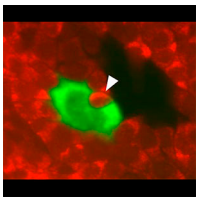
Figure S5. **Doxorubicin-induced senescent breast cancer cells engulf apoptotic Jurkat cells.** pHrodo-labeled Jurkat cells that were treated with doxorubicin for 24 h to induce apoptosis were placed onto cultures of senescent 4226 cells. Two additional representative phase and red fluorescence images of senescent 4226 cells engulfing pHrodo-labeled Jurkat cells and processing to the lysosome were captured over a time course on IncuCyte. Scale bar represents 100 µm; time of capture after addition of pHrodo Jurkats is shown. Open arrowheads indicate pHrodo Jurkats before engulfment; closed arrowheads indicated pHrodo fluorescence activation at low pH, consistent with processing to the lysosome.



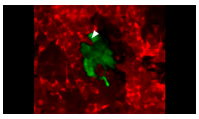
Video 1. **3D reconstruction of a GFP-expressing doxorubicin-treated senescent 4226 cell engulfing an mCherry NT 4226 cell.** Related to Fig. 2 A.



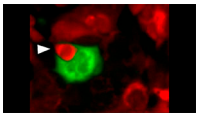
Video 2. **First example of a doxorubicin-treated senescent GFP-expressing 4226 cell engulfing an NT mCherry-expressing 4226 cell.** 49 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.



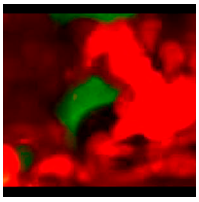
Video 3. **Second example of a doxorubicin-treated senescent GFP-expressing 4226 cell engulfing an NT mCherry-expressing 4226 cell.** 38 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.



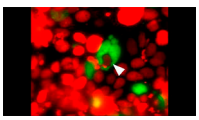
Video 4. **Third example of a doxorubicin-treated senescent GFP-expressing 4226 cell engulfing an NT mCherry-expressing 4226 cell.** 61 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.



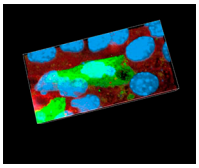
Video 5. **Fourth example of a doxorubicin-treated senescent GFP-expressing 4226 cell engulfing NT mCherry-expressing 4226 cells.** 48 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.



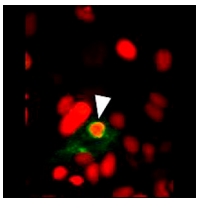
Video 6. **Doxorubicin-treated senescent GFP-expressing MCF-7 cell engulfing NT mCherry-expressing MCF-7 cells.** 31 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.



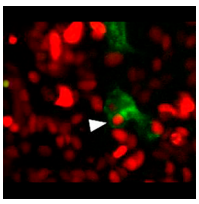
Video 7. **Doxorubicin-treated senescent H2B-GFP–expressing MCF-7 cell plated with NT H2B-mCherry MCF-7.** 93 frames, 1 frame/h. Scale bars represent 50 µm. White arrowhead marks cell to be engulfed and degraded.



Video 8. **3D reconstruction of images taken of nutlin-treated senescent 4226 cells using NIS-Elements software.** Both GFP-expressing and mCherry-expressing cells are senescent. Scale bar represents 20 µm. White arrowhead marks engulfed cell.



Video 9. **First example of a doxorubicin-treated senescent MCF-7 LAMP-1-GFP cell engulfing NT H2B-mCherry MCF-7 cells.** Representative of 16 total videos captured. 71 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.



Video 10. **Second example of a doxorubicin-treated senescent MCF-7 LAMP-1-GFP cell engulfing NT H2B-mCherry MCF-7 cells.** 71 frames, 1 frame/h. Scale bar represents 50 µm. White arrowhead marks cell to be engulfed and degraded.

Reference

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29:e45. https://doi.org/10.1093/nar/29.9.e45

Provided online are two tables. Table S1 (PDF) is a guide to supplemental videos. Table S2 (Excel) shows the statistical analysis of gene expression data in Fig. 7.