SUPPLEMENTARY FIGURES

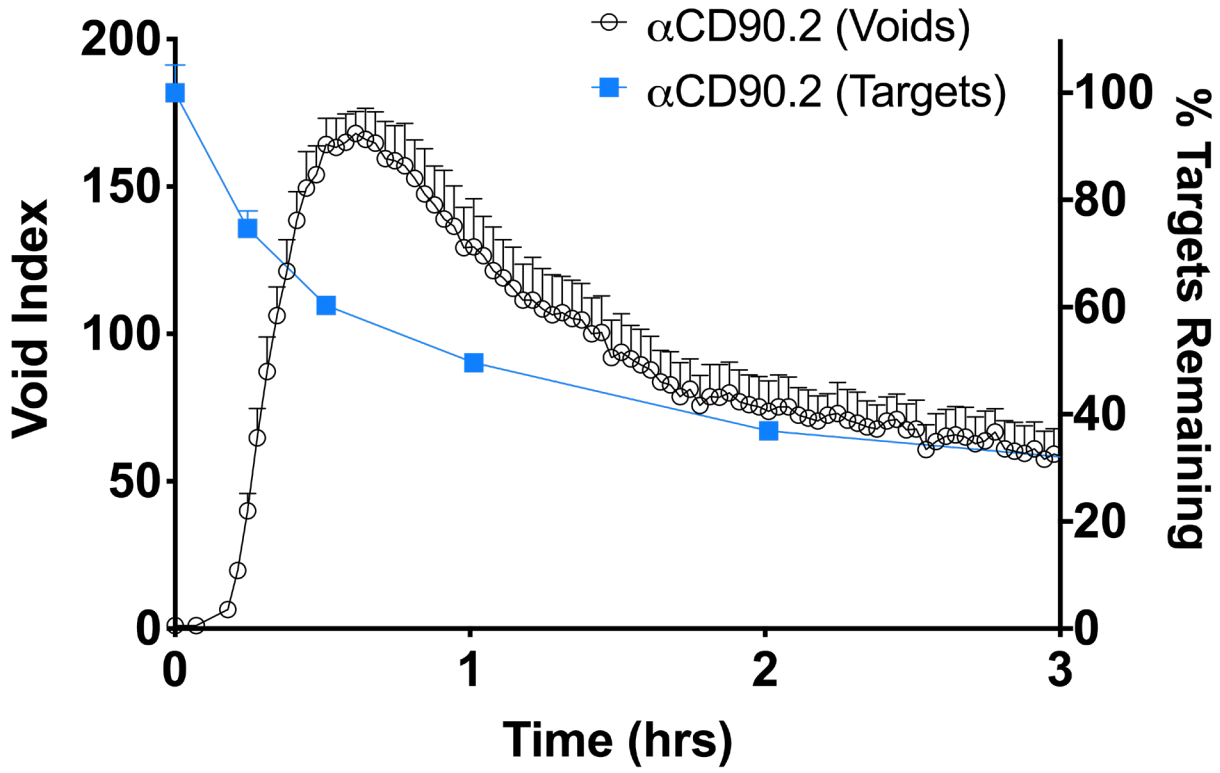


Fig. S1. Comparison of void method with validated flow cytometric cells remaining method reveals that the timing of void accumulation matches the timing of decreasing cells remaining. Void index (right y-axis) and cells remaining (left y-axis) were plotted together as a comparison to validate phagocytic quantitation by the void method (mean +s.e.m. of three independent tests).

Journal of Cell Science • Supplementary information

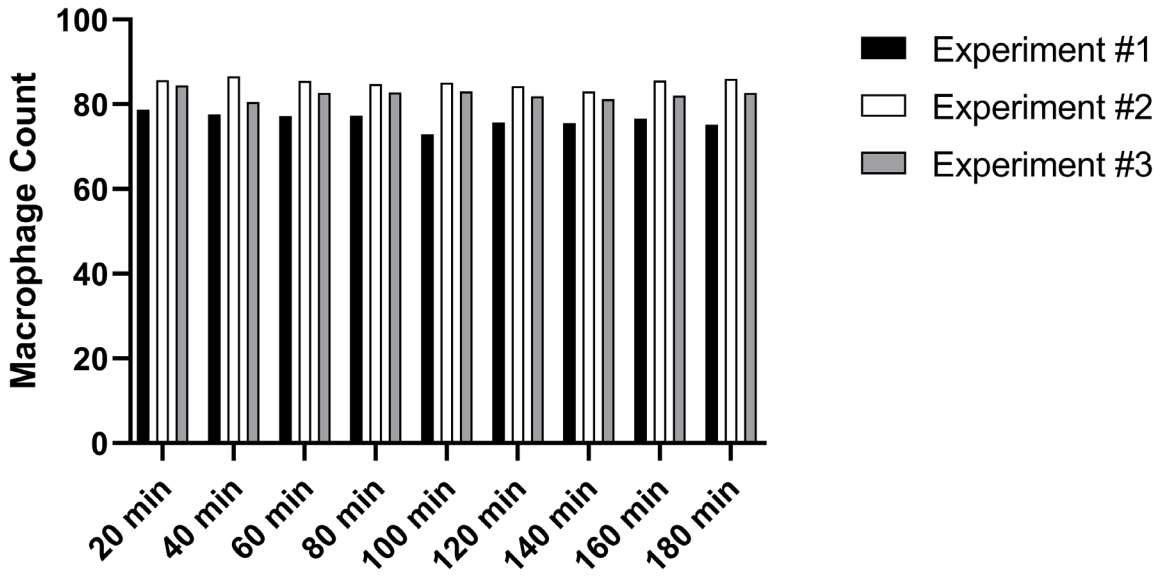


Fig. S2. Macrophage count numbers remain stable across multiple hours in multiple experiments. Bar chart showing macrophage count, or the average number of macrophages counted per field of view, within 20-minute time increments during three separate three hour experiments. Graph illustrates that macrophage counts remain largely unchanged through duration of imaging assays.

Journal of Cell Science • Supplementary information

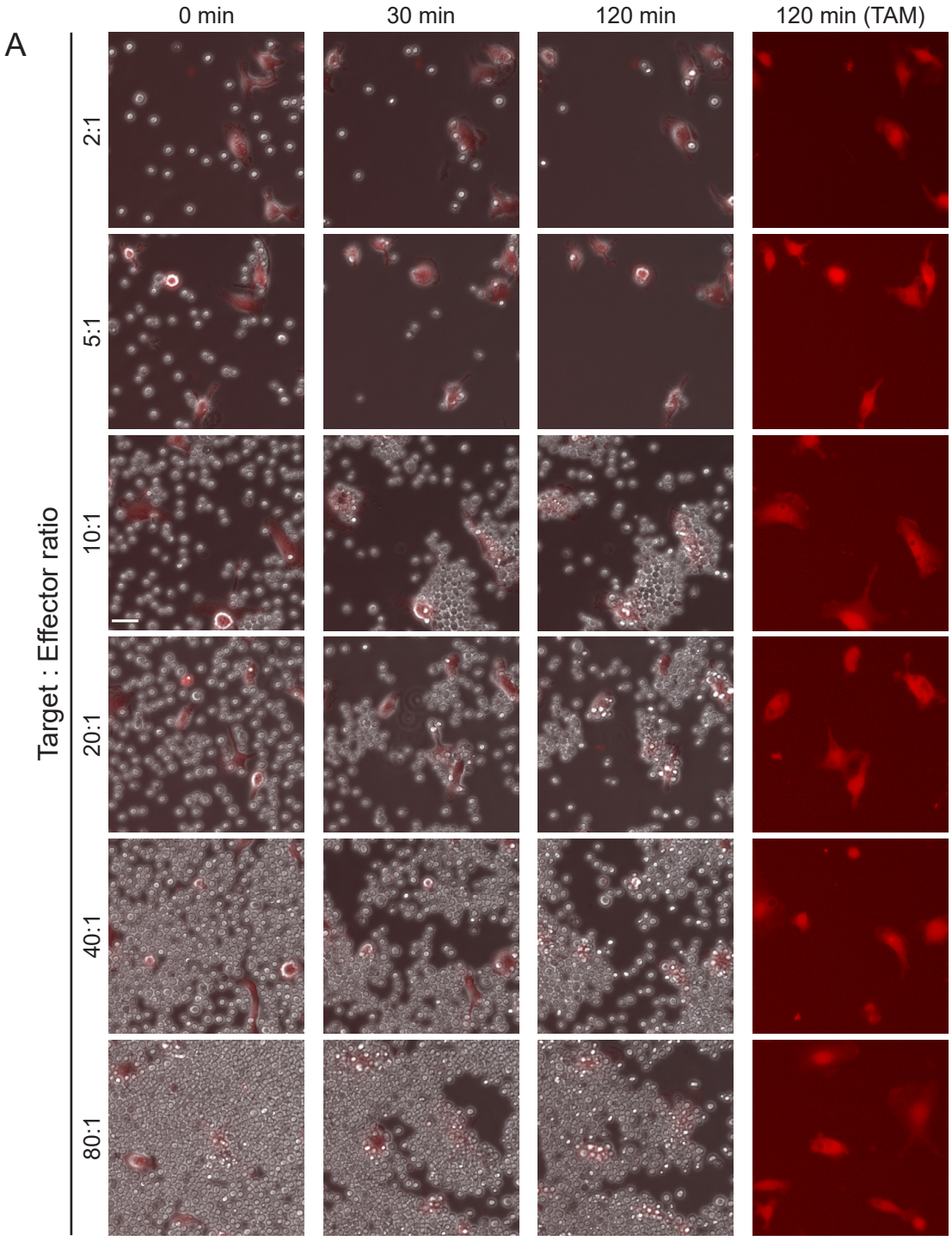


Fig. S3. Excess target cells available for phagocytosis at most target cell : macrophage effector ratios. Representative close-up images (merged phase contrast and TAMRA channels and **TAMRA channel only (right column)**) depicting target cell density at different Target to Effector cell ratios (range from 2:1 – 80:1) for three time-points across 2 hours (**Fig.3**). To ensure excess target cells in subsequent experiments, we used a 10:1 Target : Effector ratio in all remaining experiments. **Macrophages were labeled with TAMRA (red).**

Journal of Cell Science • Supplementary information

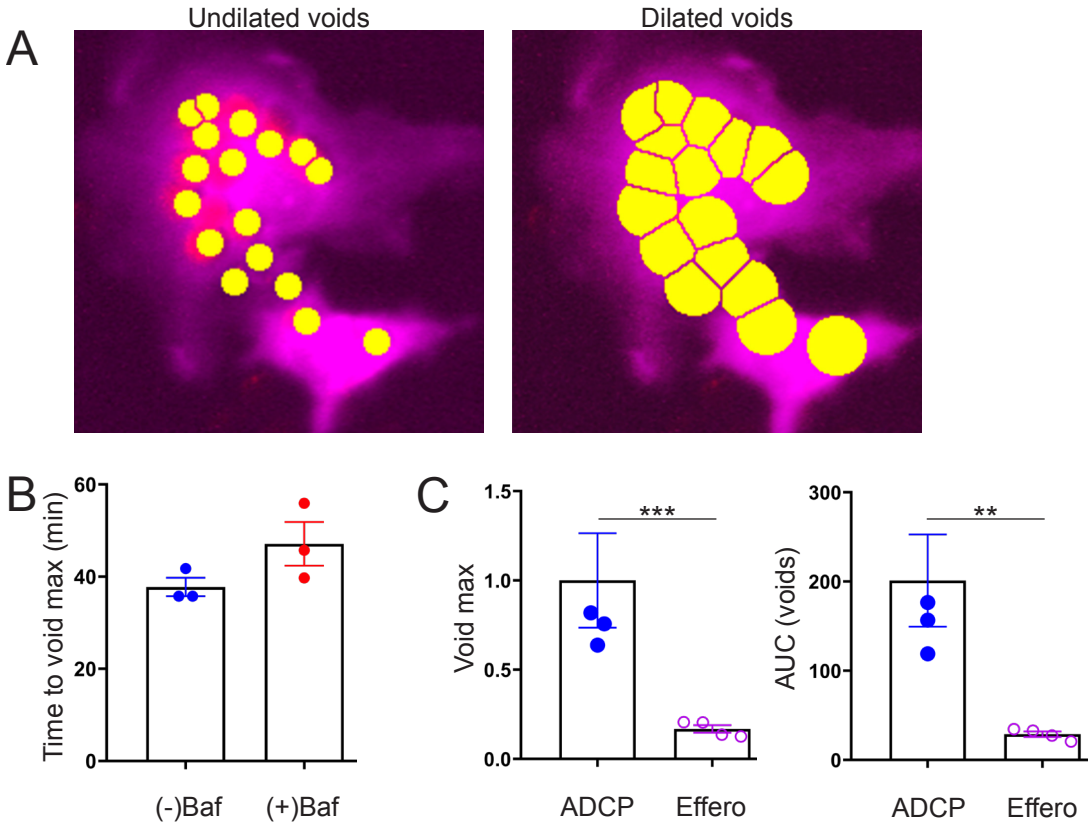


Fig.S4. Dye uptake analysis, Bafilomycin A1 effect on void index kinetics, and comparison of ADCP versus efferocytosis. (A) Dye uptake analysis uses void binary mask dilation. Representative close-up images depicting void masking before and after using the NIS-Elements software to "dilate" voids by increasing the number of pixels considered in the void mask to 30. B) Void index for anti-CD90.2 (antibody) only treated or treated with Baf (mean ±s.e.m. of three independent tests) data (**Fig.4A-E**) used to calculate measurement of time to reach maximum voids. (C) Quantitation of void index data from **Fig.4F-H** to measure overall phagocytosis by void max and AUC calculations. Data shown are mean ±s.e.m. of three independent tests. ADCP has more phagocytic activity than efferocytosis (Effero) by these measures. Statistical significance assessed by two-tailed Students t-test, *P≤0.05, ** P≤0.01. A single replicate was removed from consideration for statistical analysis as an outlier in (C) according to Grubb's test, alpha = 0.05. **Macrophages were labeled with Cell Tracker Deep Red (purple) and target cells were labeled with pHrodo Red (red).**

Journal of Cell Science • Supplementary information

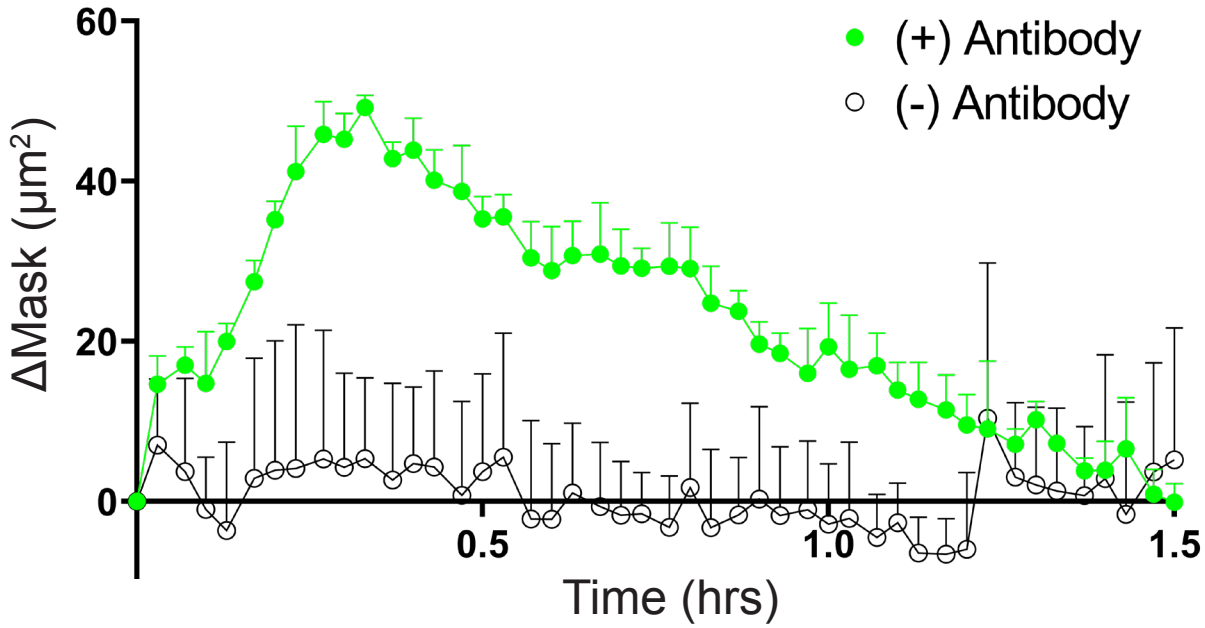
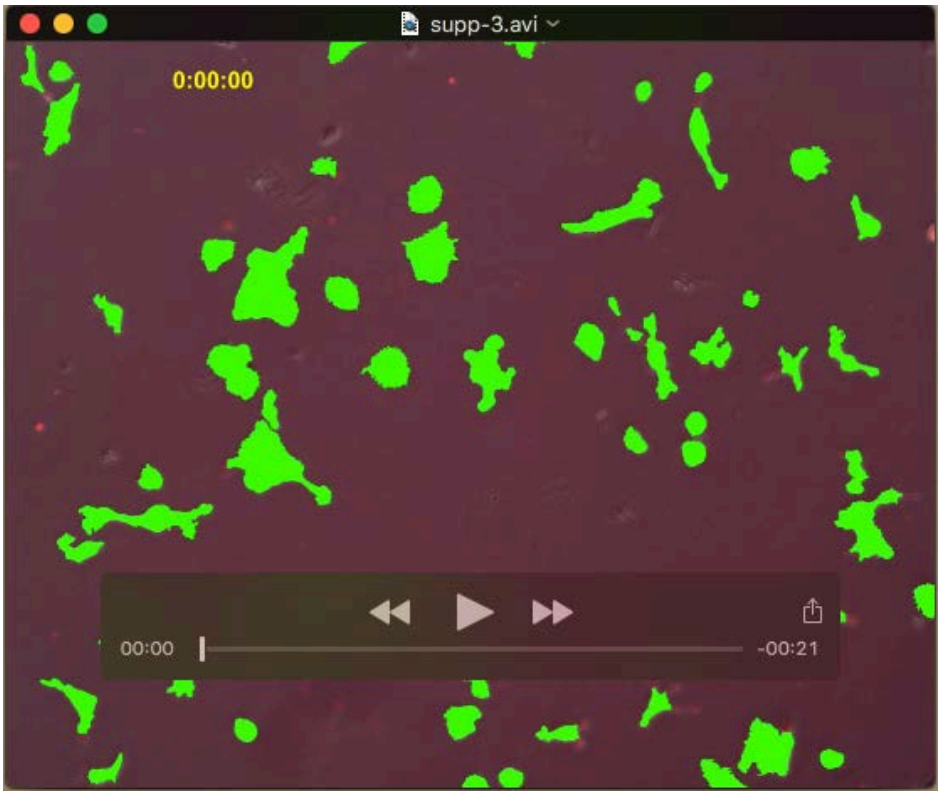


Fig.S5. Changes in observed macrophage mask parameters during ADCP is dependent on antibody. The change in macrophage mask parameters (ΔMask) was plotted from data collected in **Fig.5A-D**. Data from anti-CD90.2 mAb treated ((+) Antibody) or untreated ((-) Antibody) conditions are plotted (mean +s.e.m. of three independent tests).

Journal of Cell Science • Supplementary information

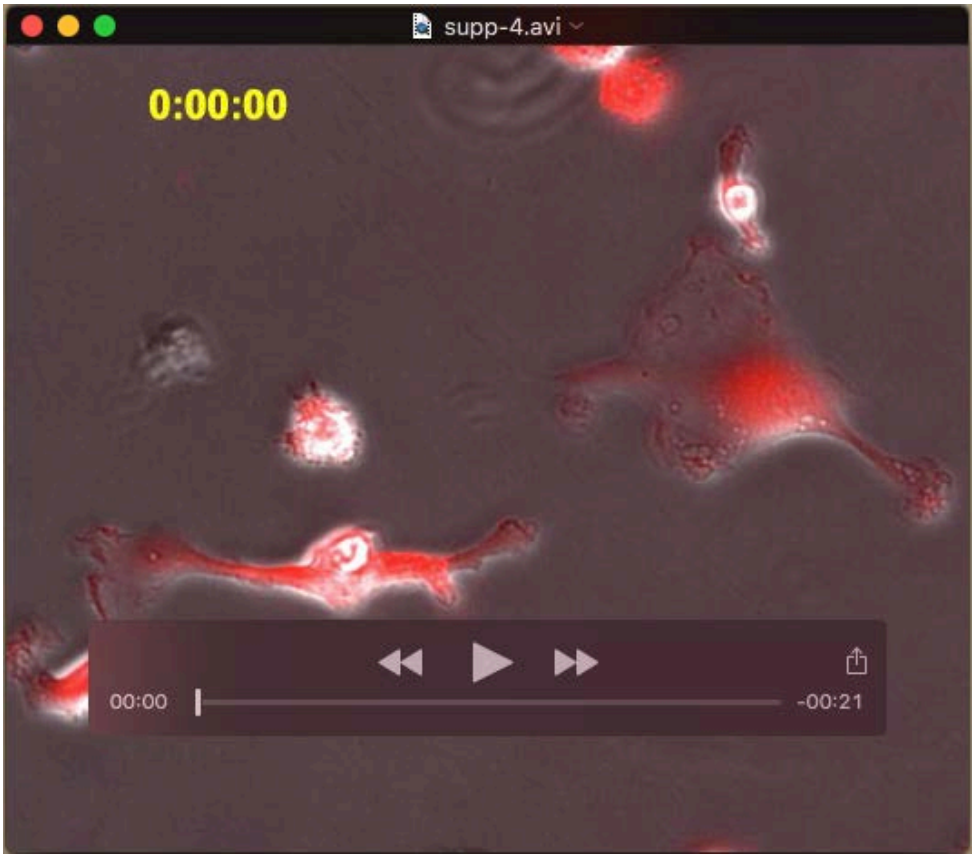
Movie 1. Video illustrates the overall complexity of phagocytosis with live cell time-lapse high-content microscopy imaging. Time-lapse video consists of 213 frames taken at 1.5 minute intervals over a duration of 5 hours and 21 minutes. Three channels, Phase contrast, TAMRA (TRITC), and CypHer5 (APC), were collected and merged. Time stamp (hr:min:sec) is shown. Mouse BMDC are labeled with TAMRA. CypHer5 -labeled target cells and anti-CD52 mAb are added at frame 8, which introduced a slight time delay and XY shift in position between frames 7 and 8. Individual frames 1 (0 min) and 40 (~60 min) are depicted in **Fig1C**.

Journal of Cell Science • Supplementary information



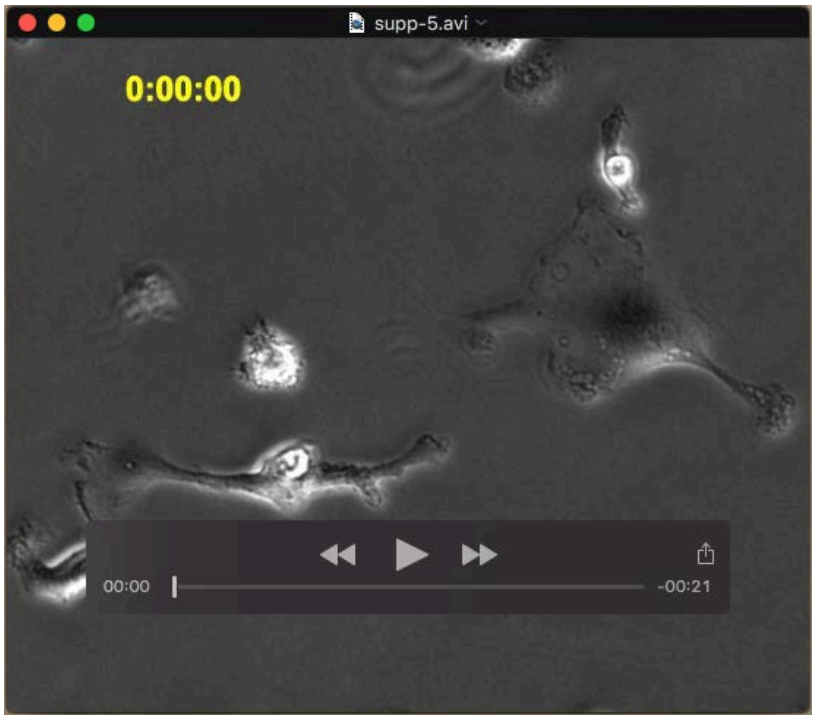
Movie 2. Video illustrates application of macrophage binary mask over the entire course of live cell time-lapse high-content microscopy imaging. Time-lapse video is the same as shown in Movie 1, but also contains an overlay of the macrophage binary mask manually determined with NIS-Elements software and then applied over all frames (green). Individual frames 1 (0 min) and 40 (~60 min) are depicted in **Fig1D**.

Journal of Cell Science • Supplementary information



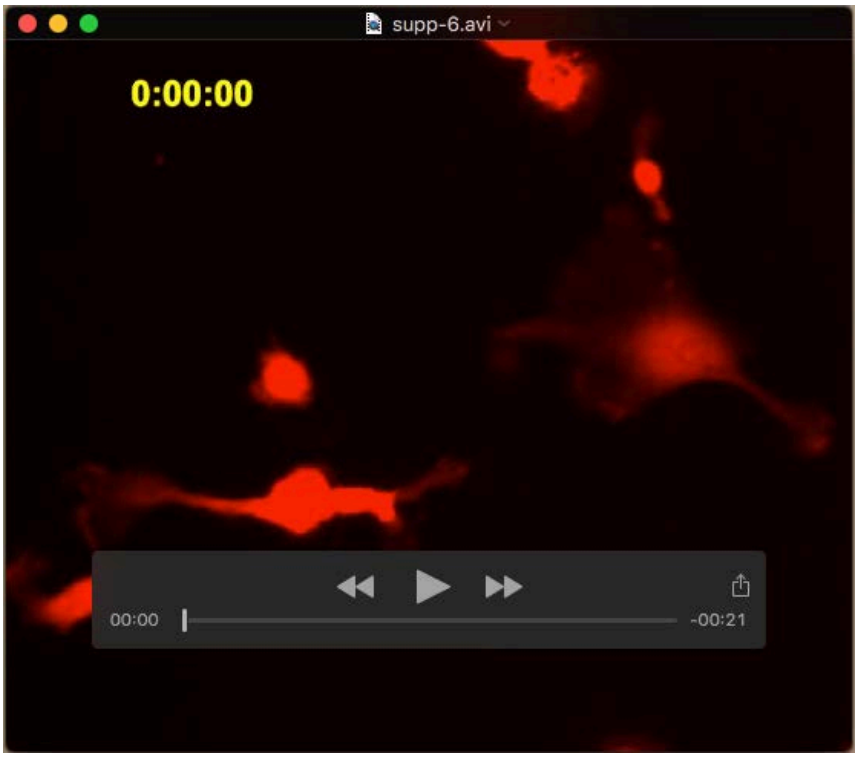
Movie 3. Video illustrates the overall complexity of phagocytosis with live cell time-lapse high-content microscopy imaging even close-up. Time lapse video is a close-up region of that shown in Movie 1. Close-up region is delineated by yellow box in **Fig.1C**. Individual frames 1 (0 min), 20 (~30 min), 40 (~60 min), and 60 (~90 min) are depicted in **Fig.2A** Merged.

Journal of Cell Science • Supplementary information



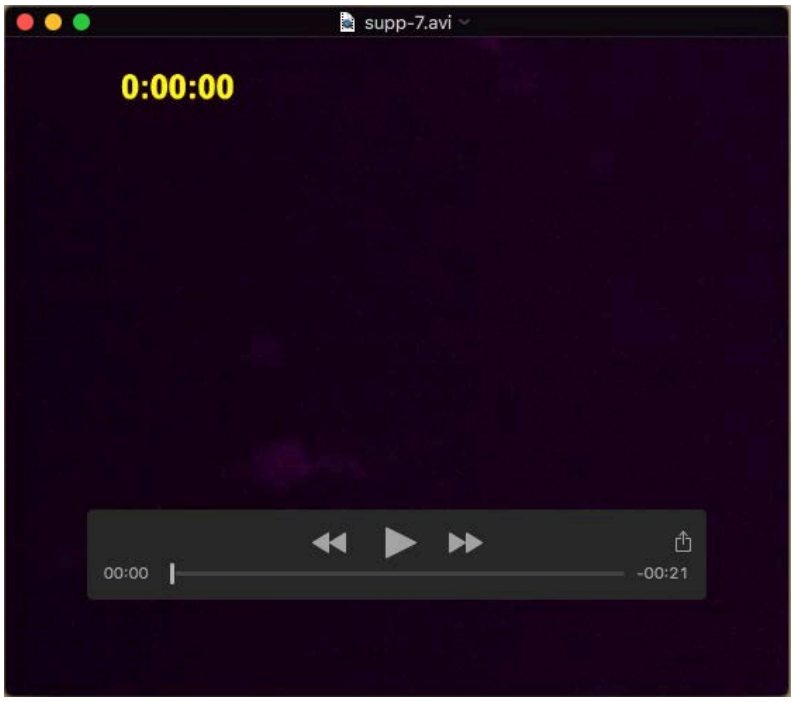
Movie 4. Video illustrates the overall complexity of phagocytosis with live cell time-lapse high-content microscopy imaging even close-up in phase contrast channel alone. Time-lapse video is same close-up region as shown in Movie 3, except only phase contrast channel is displayed. Individual frames 1 (0 min), 20 (~30 min), 40 (~60 min), and 60 (~90 min) are depicted in **Fig.2A** Phase.

Journal of Cell Science • Supplementary information



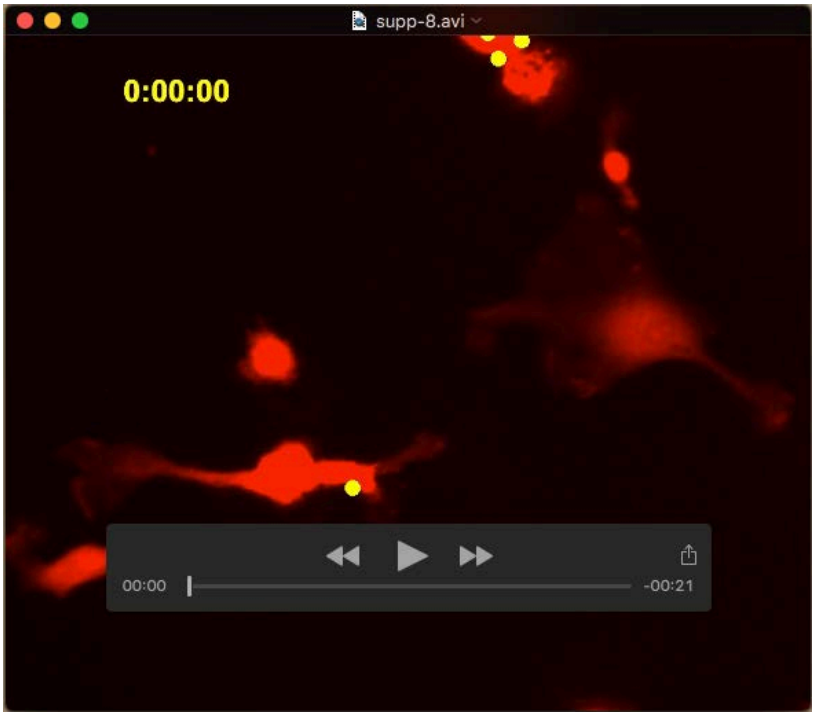
Movie 5. Video close-up illustrates reduced complexity of phagocytosis with live cell time-lapse high-content microscopy by using only macrophage dye-label channel. Time-lapse video is same close-up region as shown in Movie 3, except only TAMRA (TRITC) channel is displayed. Individual frames 1 (0 min), 20 (~30 min), 40 (~60 min), and 60 (~90 min) are depicted in **Fig.2A** TAMRA.

Journal of Cell Science • Supplementary information



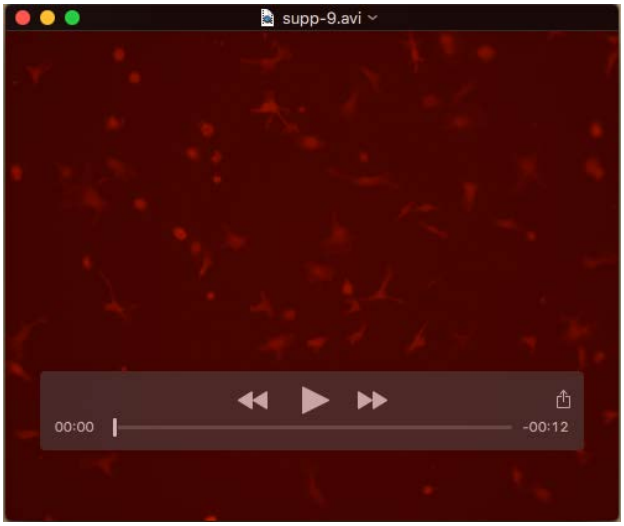
Movie 6. Video close-up of only target cell dye-label channel during phagocytosis with live cell time-lapse high-content microscopy imaging illustrates lack of initial sensitivity of dye uptake measure. Time-lapse video is same close-up region as shown in Movie 3, except only CypHer5 (APC) channel is displayed. Individual frames 1 (0 min), 20 (~30 min), 40 (~60 min), and 60 (~90 min) are depicted in **Fig.2A** CypHer5.

Journal of Cell Science • Supplementary information



Movie 7. Video close-up illustrates application of void binary mask over the entire course of live cell time-lapse high-content microscopy imaging. Time-lapse video is same close-up region as shown in Movie 3, except void binary mask (yellow) is overlaid on TAMRA (TRITC) channel display. Individual frames 1 (0 min), 20 (~30 min), 40 (~60 min), and 60 (~90 min) are depicted in **Fig.2B**.

Journal of Cell Science • Supplementary information



Movie 8. Video illustrates viability of macrophages even at highest T:E ratio over the entire course of live cell time-lapse high-content microscopy imaging. Representative time-lapse video of macrophages during an 80:1 T:E ratio experiment included in Fig.3A. TAMRA (TRITC) channel is shown for entire 4 hour experiment.

Movie 9. Video close-up illustrates the lack of target dye (pHrodo Red) intensity increase following engulfment when macrophages are pre-treated with BafA1. Time-lapse video is same close-up region as shown in Fig. 4A (Bottom) and includes the merged CTDR (macrophage) and pHrodo Red (target) channels. Movie starts at ~39 minutes due to delay between capture of background frames and addition of antibody and targets.

Journal of Cell Science • Supplementary information

Movie 10. Video close-up illustrates the representative target dye (pHrodo Red) intensity increase following ADCP engulfment. Time-lapse video is same close-up region as shown in Fig. 4F (Top) and includes the merged CTDR (macrophage) and pHrodo Red (target) channels.

Movie 11. Video close-up illustrates the representative target dye (pHrodo Red) intensity increase following efferocytosis engulfment. Time-lapse video is same close-up region as shown in Fig. 4F (Bottom) and includes the merged CTDR (macrophage) and pHrodo Red (target) channels.

Journal of Cell Science • Supplementary information