Phagocytosis of antibody-opsonized tumor cells leads to the formation of a discrete vacuolar compartment in macrophages

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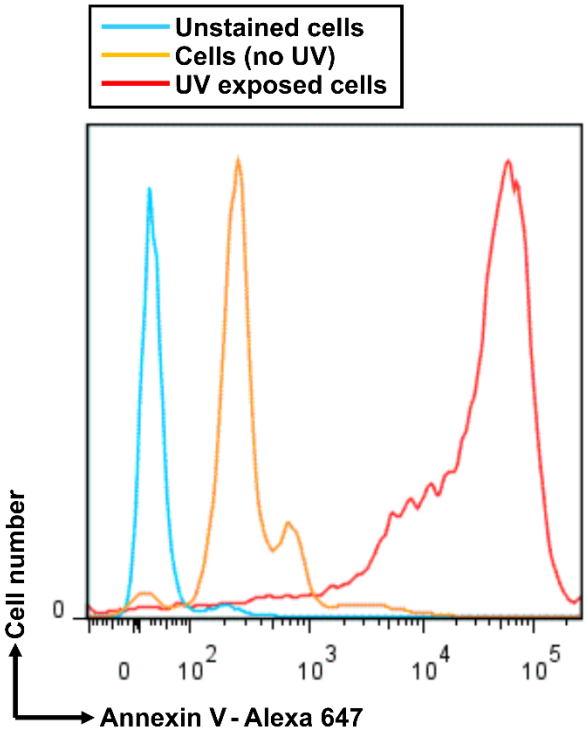
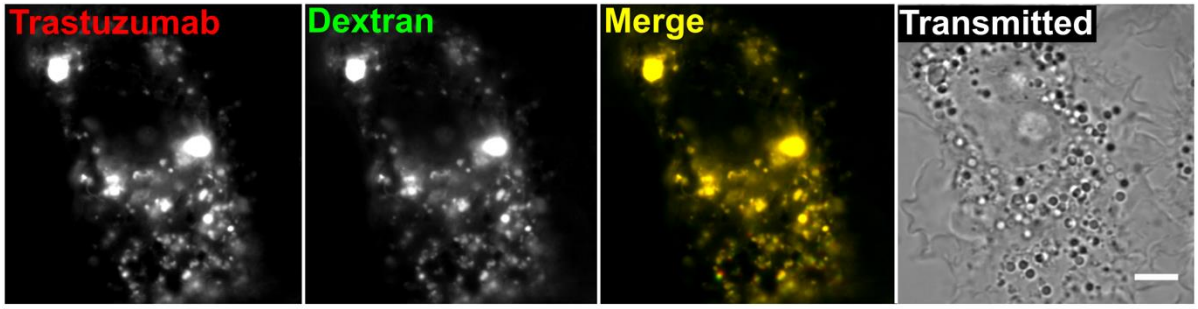
Supplemental Information

Supplementary Figures



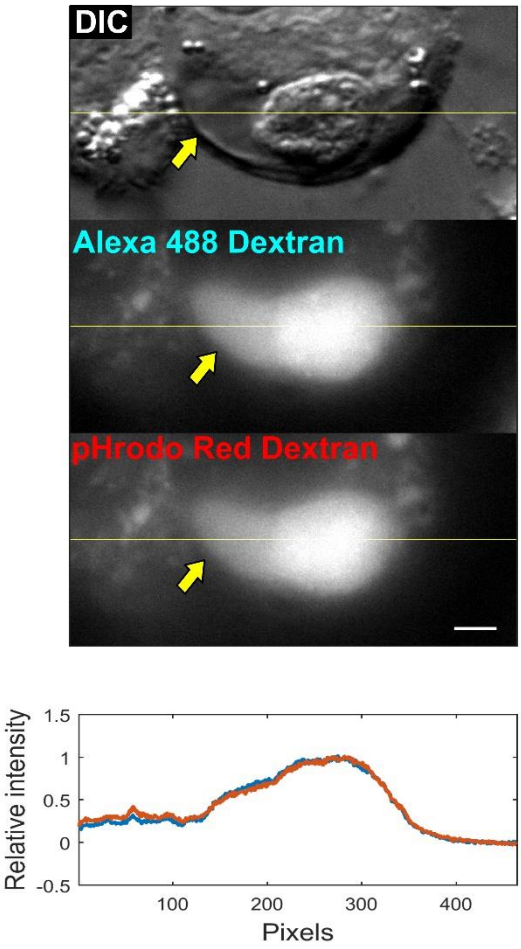
Supplementary Figure 1. Flow cytometric analyses of induction of apoptosis in MDA-MB-453 cancer cells

MDA-MB-453 cancer cells were plated overnight and UV-irradiated for 3 hours, followed by harvesting of non-adherent cells/apoptotic bodies. As a control, cells were not exposed to UV irradiation and harvested by trypsinization. Cells/apoptotic bodies were stained with Alexa 647-labeled Annexin V. Histogram plot shows flow cytometry analyses for control and apoptotic cells. Data shown are representative of two independent experiments.



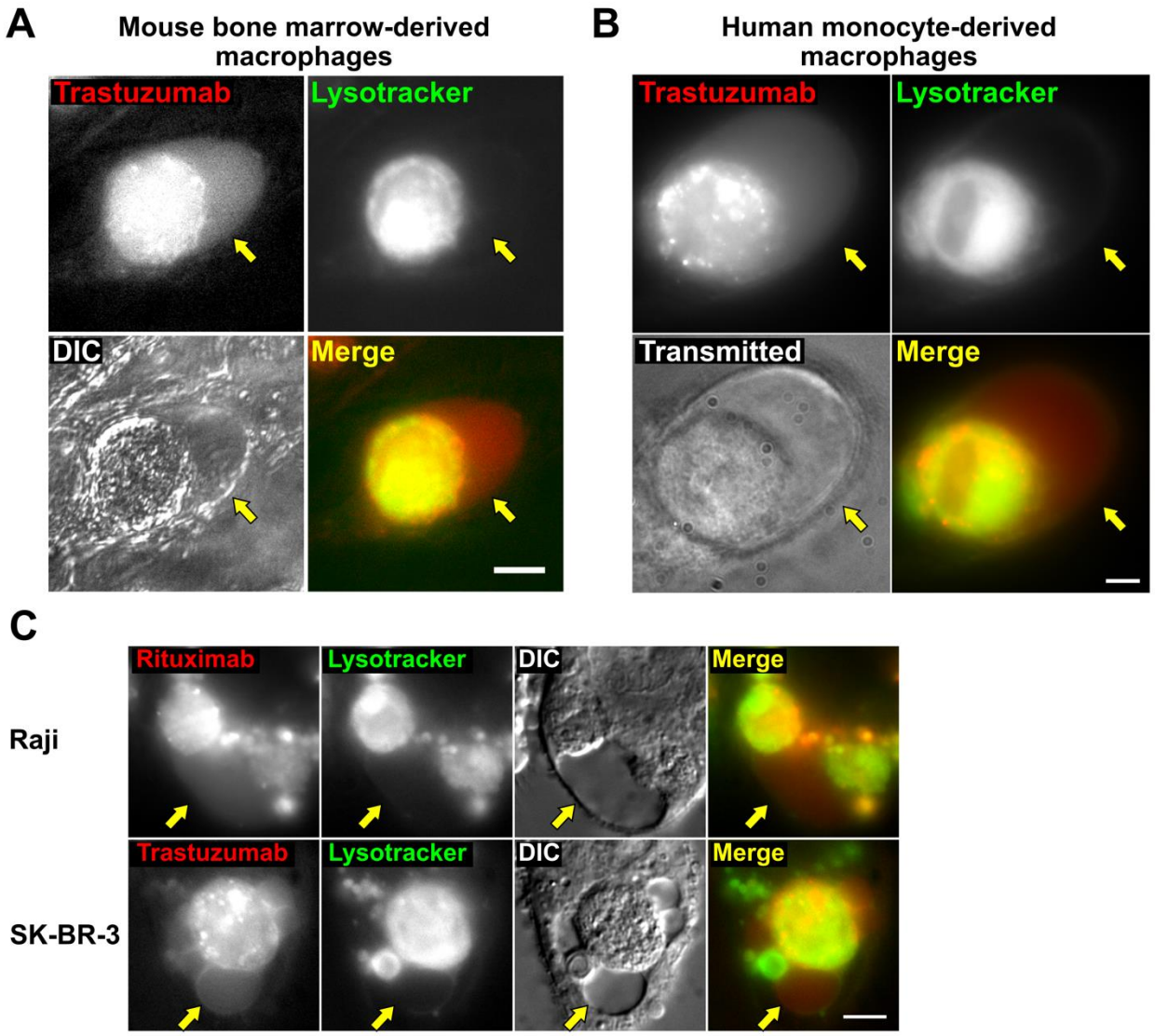
Supplementary Figure 2. Redistribution of cancer cell content into the lysosomal network of macrophages

MDA-MB-453 cells were pulse-chased (2 hours pulse, 4 hours chase) with 100 µg/ml Alexa 647-labeled 10 kDa dextran (pseudocolored green) to label lysosomes, followed by opsonization with Alexa 555-labeled trastuzumab (pseudocolored red) and co-incubation with J774A.1 macrophages for 24 hours. Fluorescent and DIC images of a representative cell from at least 16 cells and 2 independent experiments are shown. Scale bar = 5 µm.



Supplementary Figure 3. Analysis of pH in the phagosome and associated vacuole using pH-sensitive and insensitive dye conjugated dextran

J774A.1 macrophages preloaded with dextran were co-incubated with MDA-MB-453 cancer cells opsonized with trastuzumab as described in the legend for Fig. 3E. Fluorescent and DIC images are shown, with a line drawn across the phagosome/vacuole. Line intensity plot represents the normalized intensity for the two fluorescent signals (Alexa 488 and pHrodo Red, shown in blue and red, respectively) detected along the yellow line. Data for each fluorophore are normalized against the maximum signal level. The ratio of fluorescent intensities in the phagosomes and vacuoles were quantitated and 40% (n = 110) of vacuoles were found to have similar pHrodo Red:Alexa 488 intensity ratios in both the vacuole and adjacent phagosome. Yellow arrows indicate the location of the vacuole, and images of a representative cell from 110 cells and 3 independent experiments are shown. Scale bar = 5 µm.



Supplementary Figure 4. Phagosome-associated vacuoles are observed with multiple effector and target cell types

A, MDA-MB-453 cells opsonized with Alexa 488-labeled trastuzumab (pseudocolored red) were co-incubated with mouse bone marrow-derived macrophages for 6 hours, followed by addition of Lysotracker Red (pseudocolored green) and imaging (fluorescence and DIC). B, MDA-MB-453 cells opsonized with Alexa 647-labeled trastuzumab (pseudocolored red) were co-incubated with human monocyte-derived macrophages for 6 hours and treated/analyzed in for panel A. C, Raji B cells opsonized with Alexa 647-labeled rituximab or SK-BR-3 cells opsonized with Alexa 488-labeled trastuzumab (pseudocolored red) were co-incubated with J774A.1 macrophages for 4.5 hours or 6 hours, respectively, and treated/analyzed as in panel A. Yellow arrows in A-C indicate the location of the vacuole. Images of representative cells from at least 34 cells and 2 independent experiments are shown. Scale bars = 5 μm.

Supplementary movie legends

Movie S1. Formation of the phagosome-associated vacuole

The movie corresponds to Figure 1A. Time-lapse images of transmitted light (left), Alexa 647-labeled dextran preloaded in lysosomes of J774A.1 macrophages (center), and Alexa 555-labeled trastuzumab originating from opsonized MDA-MB-453 cells (right) are shown. Time on the upper left is shown in the hours:minutes:seconds format. The movie plays at a speed of 1500x real-time. Scale bar = 5 μm.

Movie S2. Redistribution of the contents of the phagosome and the associated vacuole

The movie corresponds to Figure 2C. Time-lapse images of Alexa 488-labeled dextran preloaded in lysosomes of J774A.1 macrophages (upper left, pseudocolored red), Alexa 555-labeled dextran preloaded in lysosomes of MDA-MB-453 cancer cells (lower left, pseudocolored green), and DIC (upper right) are shown. Time in the upper right panel is shown in the hours:minutes format. The movie plays at a speed of 2600x real-time. Scale bar = 5 μm.