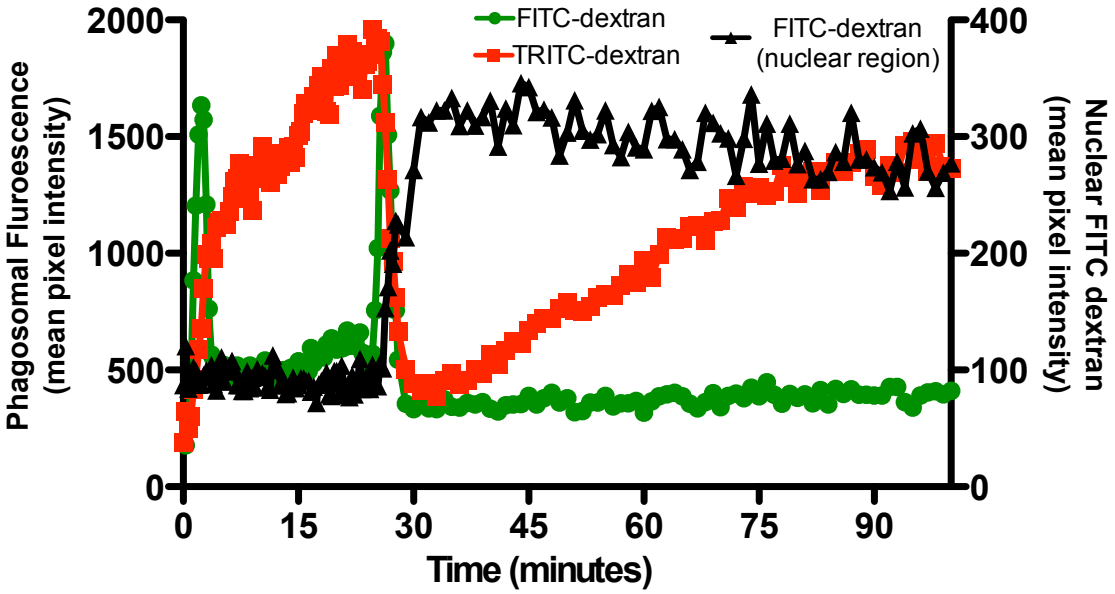
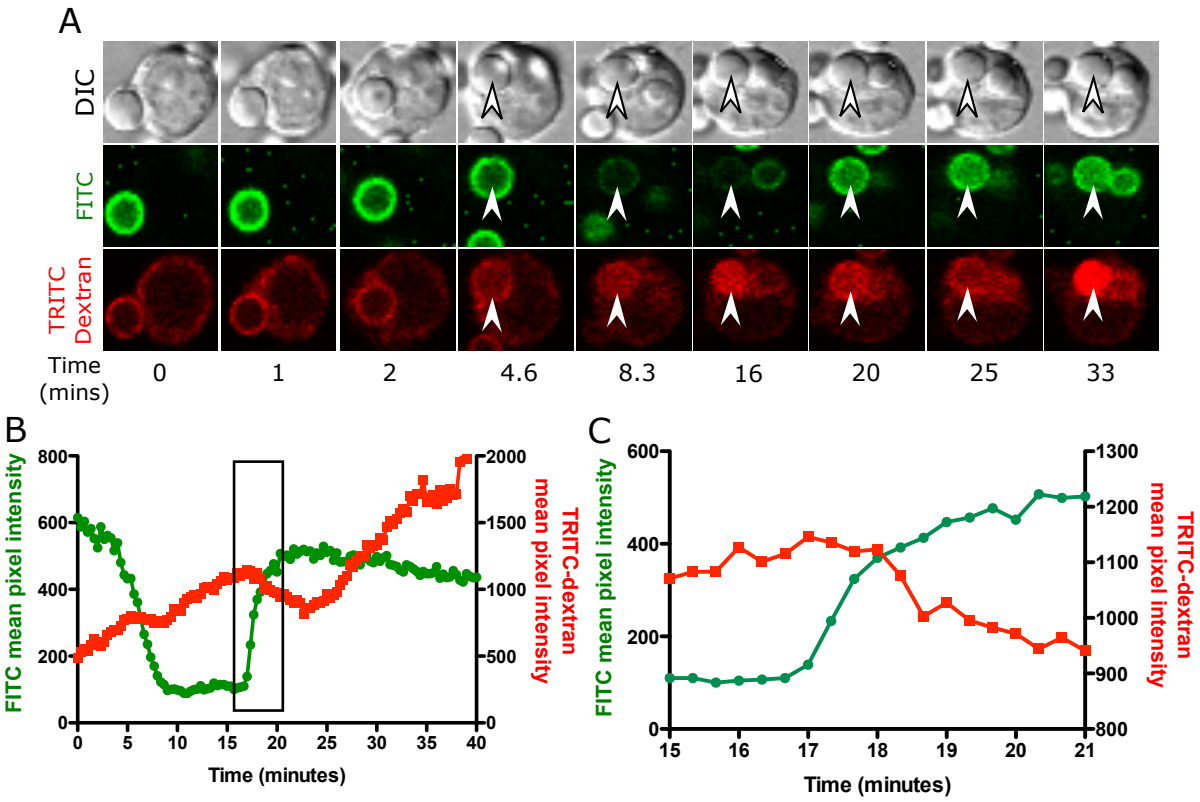
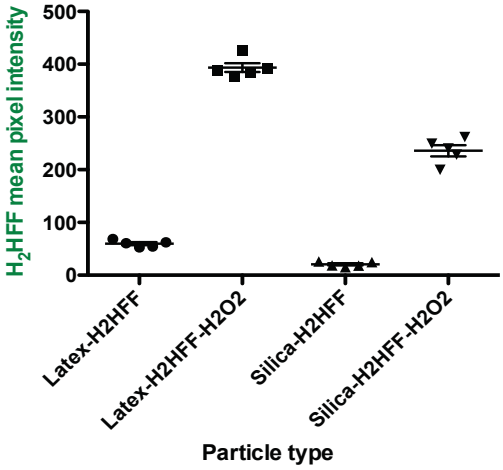
Supplemental Materials

Molecular Biology of the Cell

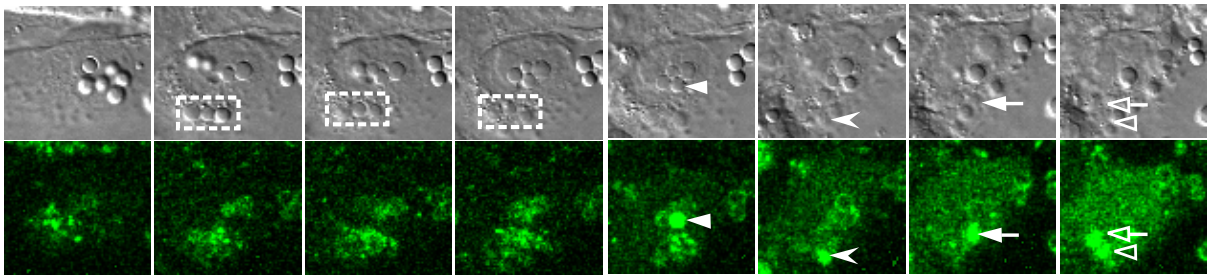
Joshi et al.


FITC-dextran
TRITC-dextran
FITC-dextran
(nuclear region)
Phagosomal Fluroescence
(mean pixel intensity)
Nuclear FITC dextran
(mean pixel intensity)
2000
1500
1000
500
0
400
300
200
100
0
0
15
30
45
60
75
90
Time (minutes)


A
DIC
FITC
TRITC Dextran
Time (mins)
0
1
2
4.6
8.3
16
20
25
33
B
FITC mean pixel intensity
TRITC-dextran mean pixel intensity
Time (minutes)
C
FITC mean pixel intensity
TRITC-dextran mean pixel intensity
Time (minutes)

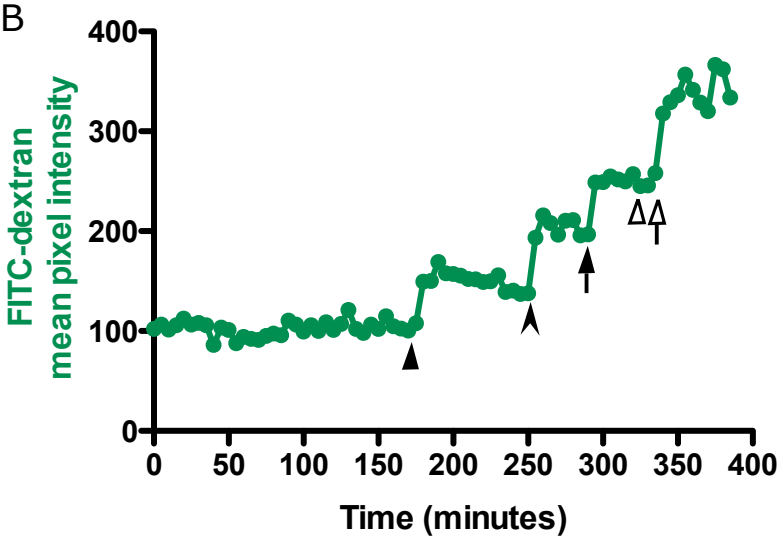

500
400
300
200
100
0
H2HFF mean pixel intensity
Latex-H2HFF
Latex-H2HFF-H2O2
Silica-H2HFF
Silica-H2HFF-H2O2
Particle type

A

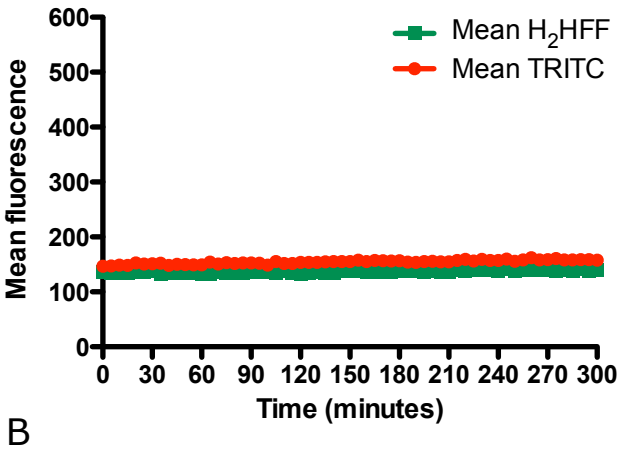


Time (mins) 0 135 140 145 180 255 300 340

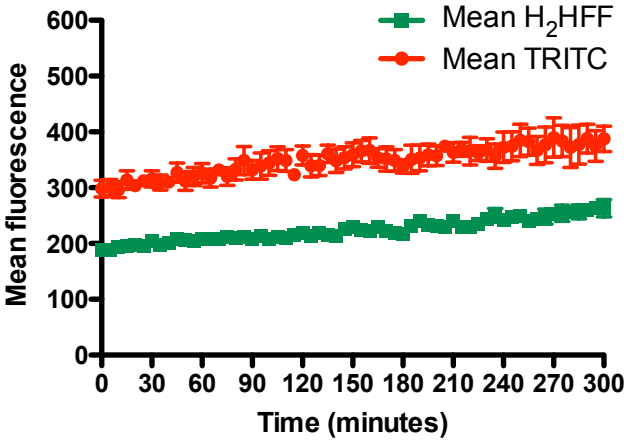
B



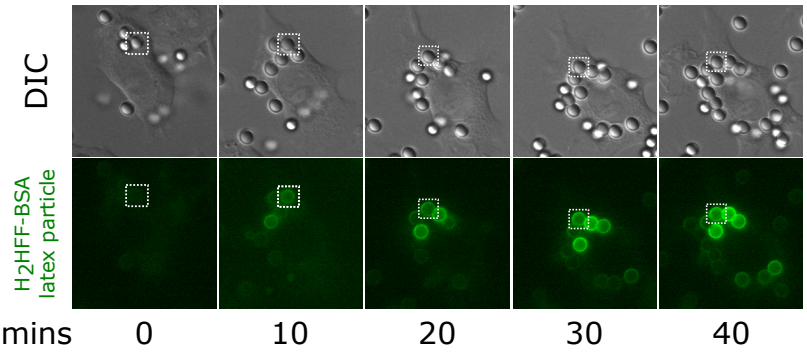
A


Mean H_2HFF
Mean TRITC
Mean fluorescence
600
500
400
300
200
100
0
0 30 60 90 120 150 180 210 240 270 300
Time (minutes)

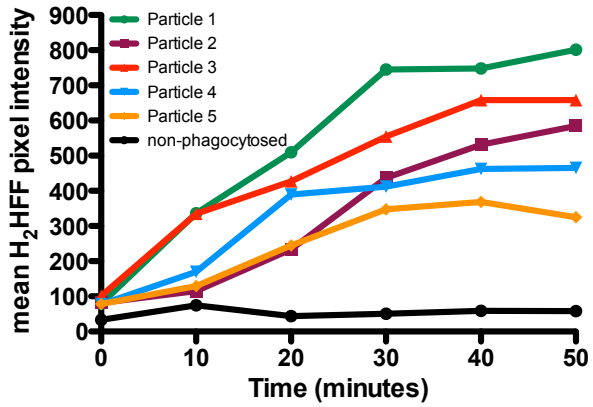
B


Mean H_2HFF
Mean TRITC
Mean fluorescence
600
500
400
300
200
100
0
0 30 60 90 120 150 180 210 240 270 300
Time (minutes)

A


DIC
H2HFF-BSA latex particle
mins
0
10
20
30
40

B


mean H2HFF pixel intensity
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
Time (minutes)
Particle 1
Particle 2
Particle 3
Particle 4
Particle 5
non-phagocytosed

Supplementary Figure 1
Representation of data set in Figure 1
The entire data set from Figure 1 is presented in this figure. A continuous increase in TRITC-dextran fluorescence until 24 minutes was seen indicative of continuous vesicle fusion. Following phagolysosomal leakage a gradual increase in TRITC-dextran fluorescence from 32 min onwards was seen.

Supplementary Figure 2
Fluorescence changes following uptake of silica particles directly labeled with FITC
Opsonized silica particles were labeled with FITC and added to MH-S macrophages loaded with TRITC-dextran. Five z-slices at 2 µm intervals were collected every 20 seconds. A representative particle is shown. (A) DIC images show a cell-particle interaction (0 minute) following which cell membrane extends around the particle to form a phagocytic cup (1 minute) resulting in particle phagocytosis (2 minutes). A gradual decrease in FITC fluorescence and an increase in TRITC-dextran fluorescence was seen from 2 minutes onwards. By 8 minutes FITC fluorescence is quenched and TRITC-dextran fluorescence is localized throughout the volume of the silica particle rather than the rim due to protein proteolysis. FITC fluorescence increases at 20 minutes and simultaneously a decrease in TRITC-dextran fluorescence is observed due to phagolysosomal leakage. During resealing of a leaky phagolysosome an increase in TRITC-dextran fluorescence is again observed at 33 minutes. (B) Quantification for the particle in panel A reveal temporal changes in FITC and TD fluorescence. An initial decrease in FITC fluorescence is due to phagosomal acidification where as upon leakage the fluorescence again increases. TRITC-dextran fluorescence increases initially during phagosome maturation, decreases during phagolysosomal leakage and again increases during resealing. (C) A zoom of the area highlighted in rectangular box in B shows a temporal gap between phagolysosomal leakage beginning at 17 minutes as indicated by an increase in FITC fluorescence and a release of TRITC-dextran beginning at 18 minutes.

Supplementary Figure 3
Testing sensitivity of H_2HFF labeled silica particles upon addition of exogenous H_2O_2
(A) H_2HFF labeled opsonized latex and silica particles were tested for their sensitivity to oxidation by imaging them after addition of 0.5 M H_2O_2. This control was used to determine parameters for imaging the particles in cells to measure changes in H_2HFF fluorescence. A 3-4 fold increase in fluorescence was observed with latex and silica particles.

Supplementary Figure 4
Cos7 cells show a step-wise increase in FITC-dextran fluorescence upon phagolysosomal leakage
Cos7 cells were allowed to endocytose 4 kD FITC-dextran following which they were exposed to non-opsonized silica particles and imaged 5 minutes apart using a confocal microscope. (A) The time-point preceding the uptake of the first particle is set as time 0. In a flat cell like Cos7 particle uptake can be determined by changes in the refraction

property of the particle. In a DIC image, a particle that is not internalized has a strong shadow on one side (particles in dotted rectangular area at 135 minutes). Upon particle uptake the intensity of the shadow decreases (particles in dotted rectangular area at 145 minutes). A quantal release of FITC-dextran from individual phagolysosomes due to leakage results in an increase in nuclear and cytoplasmic FITC-dextran fluorescence. A transient increase in FITC-dextran fluorescence was observed in each of these phagolysosomes (indicated by solid triangle at 180 minutes, arrowhead at 255 minutes, arrow at 300 minutes and an open triangle and open arrow at 340 minutes). This is suggestive of an increase in pH due to permeability of the phagolysosomal membrane. An increase in cytoplasmic and nuclear fluorescence is observed along with this transient increase in phagolysosomal FITC-dextran fluorescence. (B) Quantification of cytoplasmic and nuclear area show a stepwise increase in FITC-dextran fluorescence during leakage events as indicated by a solid triangle, arrowhead, arrow and an open triangle and an arrow that correspond to panel A.

Supplementary Figure 5
Particles labeled with H_2HFF that are not phagocytosed do not undergo photo-oxidation.
(A) The fluorescence intensity of H_2HFF and TRITC-dextran labeled silica particles that were used in Figure 7 A-C but not taken up by a Cos7 cells does not change over time. While TRITC-dextran fluorescence would not be expected on external particles, we have found that the particles acquire low, but measurable, TRITC fluorescence when exposed to cells labeled with this dye. (B) Fluorescence intensity of H_2HFF and TRITC on a latex particle that was not taken up by a Cos7 cells does not change significantly over time.

Supplementary Figure 6
Macrophages exposed to non-opsonized latex particles also result in phagosomal ROS
A) MH-S macrophages were exposed to H_2HFF labeled non-opsonized latex particles and imaged 10 minutes apart. An increase in fluorescence of multiple particles is observed over time (B) Quantification of these latex particles show a variation in the oxidation of the dye. Particle highlighted by square in A) is represented as particle 1 where as other particles are labeled from 2-5.