Supplemental material

Figure S1. Streptavidin-coated polystyrene beads were opsonized with different amounts of Texas Red-conjugated anti-streptavidin (IgG). Different IgG amounts on bead surfaces were measured by widefield fluorescence microscopy and normalized. Texas Red fluorescence per bead was measured as a function of Texas Red-IgG concentration during opsonization.

Figure S2. YFP-AktPH was recruited to phagosomes with similar magnitudes for B1 and B10. Time-lapse, phase-contrast and Re images of YFP-AktPH transfected RAWs internalizing B10 (**A**) and B1 (**B**) are shown at 2-minute intervals. The arrowheads show that B10 recruited similar amounts of YFP-AktPH with B1. Scale bar is 5 μm. (**C**) Stalled phagocytic cups also recruited YFP-AktPH, but to a lower magnitude than finished phagosomes.

Figure S3. BtkPH-YFP and YFP-Tapp1PH were recruited to phagosomes with similar magnitudes for B1 and B10. The average recruitment of BtkPH-YFP (mean ± s.e.m., n=11) (**A**) and YFP-Tapp1PH (n=9) (**C**) per pixel in phagosomes of B10 (red) and B1 (blue) were plotted as a function of time, aligned by the start of membrane movement for phagocytosis. The average maximum BtkPH-YFP (**B**) and YFP-Tapp1PH (**D**) recruitment during completed phagocytic events showed no difference between B10 and B1.

Figure S4. YFP-AktPH recruitment exceeds the threshold level before phagosome closure. The timing of YFP-AktPH recruitment to cups relative to the time of phagosome closure. During complete phagocytosis of both B1 and B10, YFP-AktPH recruitment exceeded threshold 4-5 minutes before phagosome closure, indicating 3'PI accumulation was not a consequence of phagosome sealing (n=8).

Figure S1

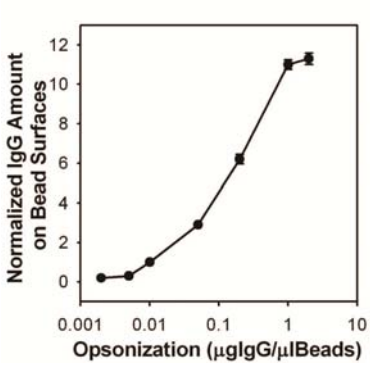


Figure S2

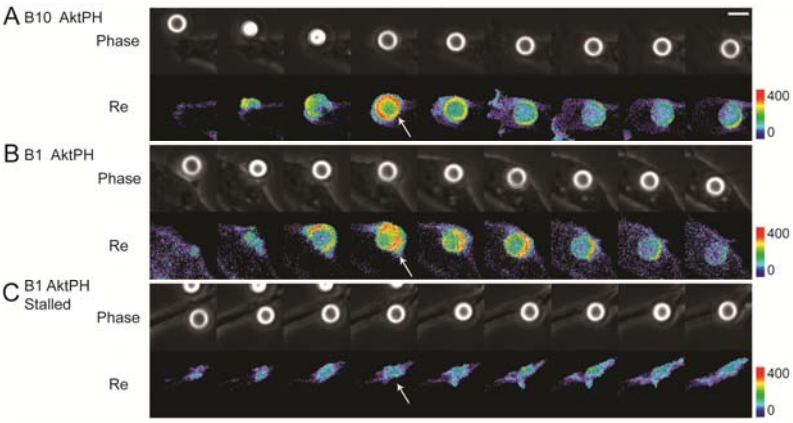


Figure S3

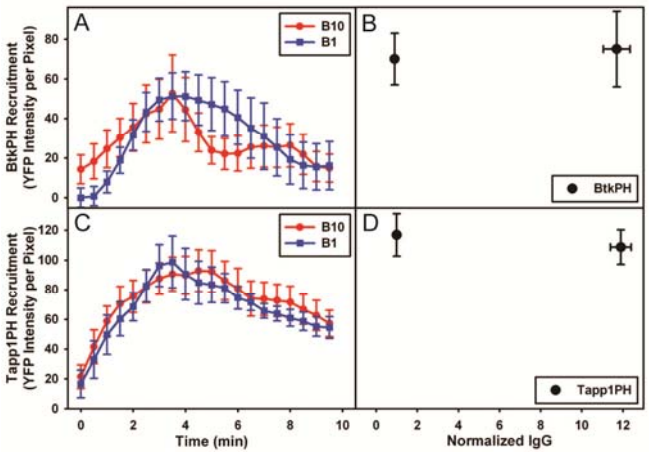
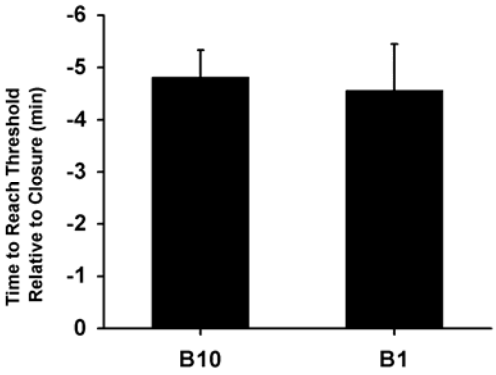


Figure S4


Time to Reach Threshold
Relative to Closure (min)
-6
-5
-4
-3
-2
-1
0
B10
B1