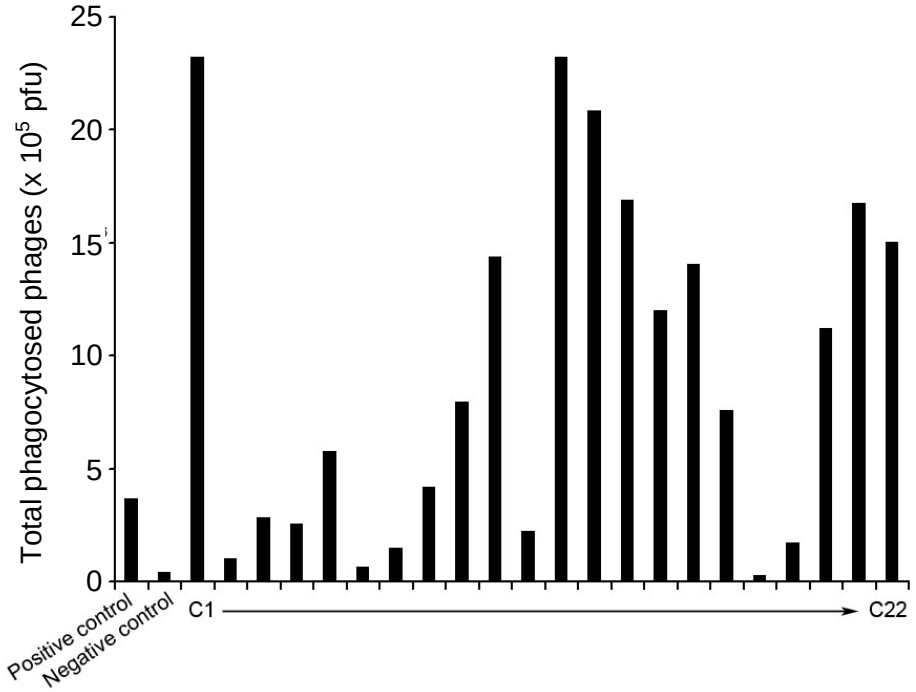
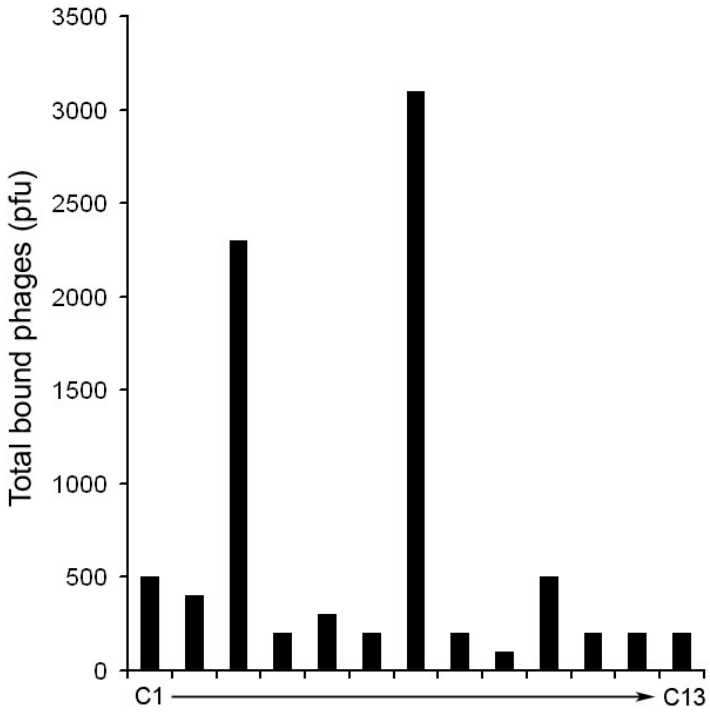
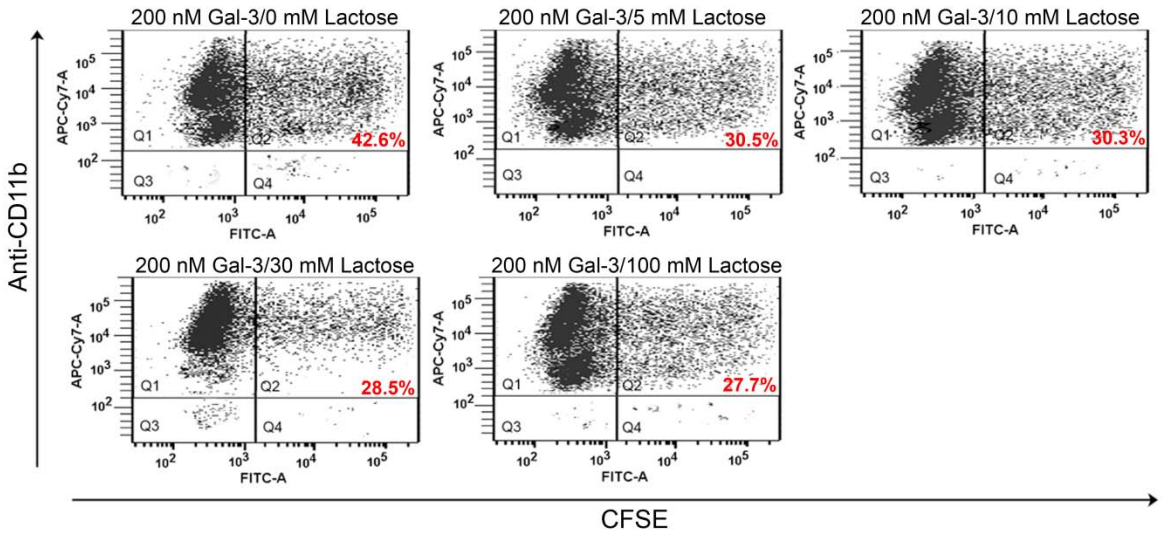


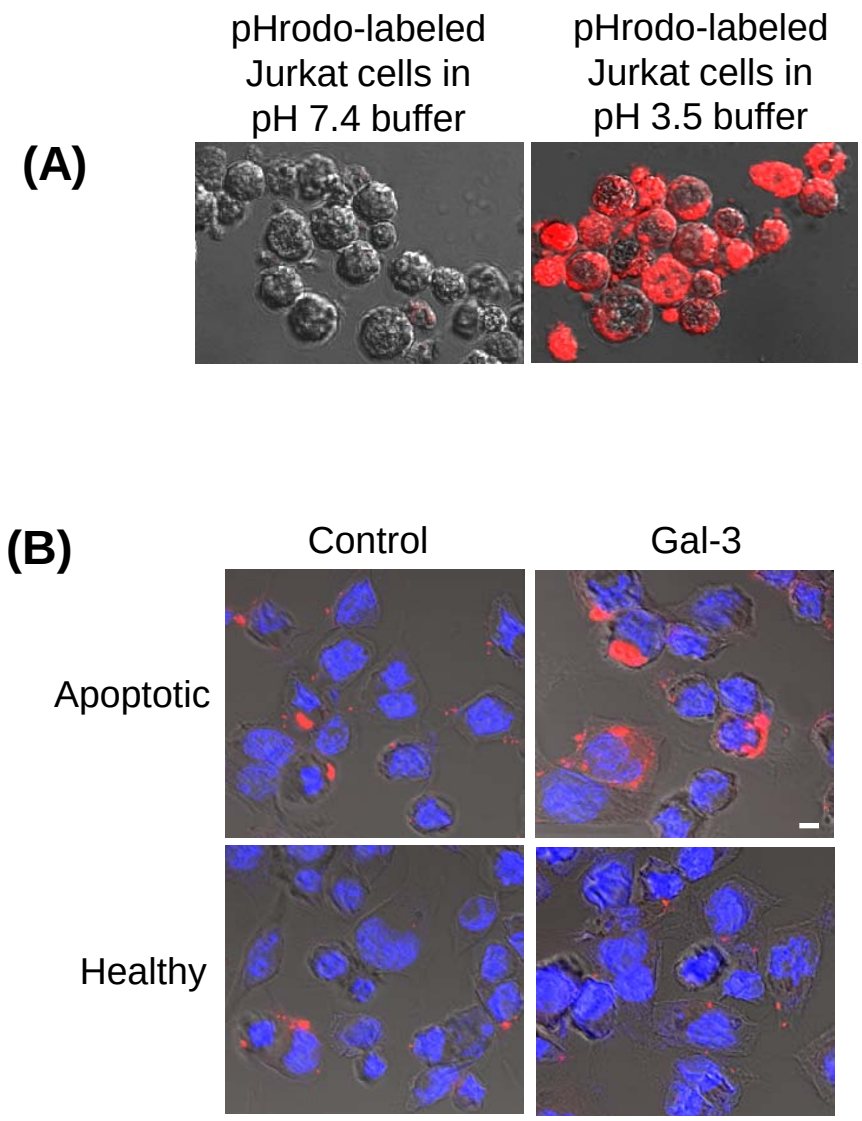
Supplementary Figure 1. Functional screening for phagocytosis ligands. The ORF phage display cDNA library was enriched by 4 rounds of dual functional selection. Enriched clones were randomly picked from phage plates, amplified and analyzed for their phagocytosis activity in D407 cells, as described (Caberoy et al., 2010a). Total phagocytosed phages were quantified by plaque assay. The control phage without foreign cDNA insert was included as a negative control. The enriched phages at round 4 were included as a positive control. A total of 96 clones were screened. Only the first 22 clones are shown here.



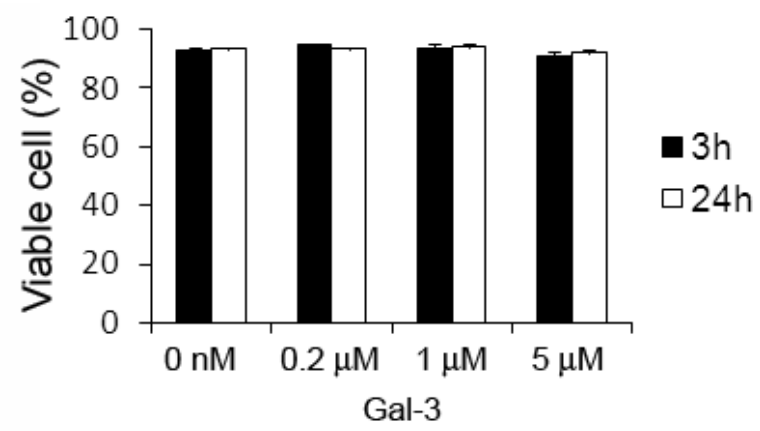
Supplementary Figure 2. Screening for MerTK-binding ligands. Positive phage clones from Supplementary Figure 1 were amplified and analyzed for their binding to immobilized Mer-Fc on ELISA plates by phage binding assay. After washing, bound phages were eluted by 3C protease cleavage (Caberoy et al., 2010b) and quantified by plaque assay. A total of 43 clones were analyzed by the receptor-binding assay. Only the first 13 were shown here.



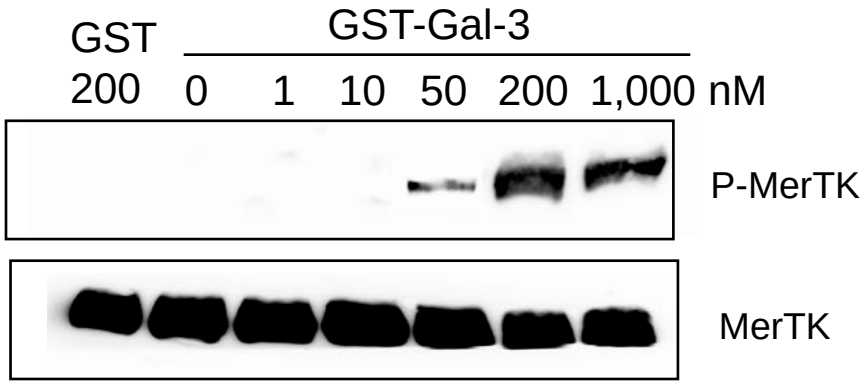
Supplementary Figure 3. Dose-dependent blockade of Gal-3-mediated macrophage phagocytosis by lactose. Phagocytosis of CFSE-labeled apoptotic Jurkat cells by J774 macrophages was analyzed in the presence of purified GST-Gal-3 (200 nM) and increasing concentrations of lactose. The macrophages were treated with trypsin to remove surface-bound Jurkat cells, washed, labeled with APC/Cy7-conjugated anti-CD11b for macrophages and analyzed by flow cytometry, as described in Fig. 3C. The number in each panel indicates the percentage of J774 cells with phagocytosed apoptotic Jurkat cells.



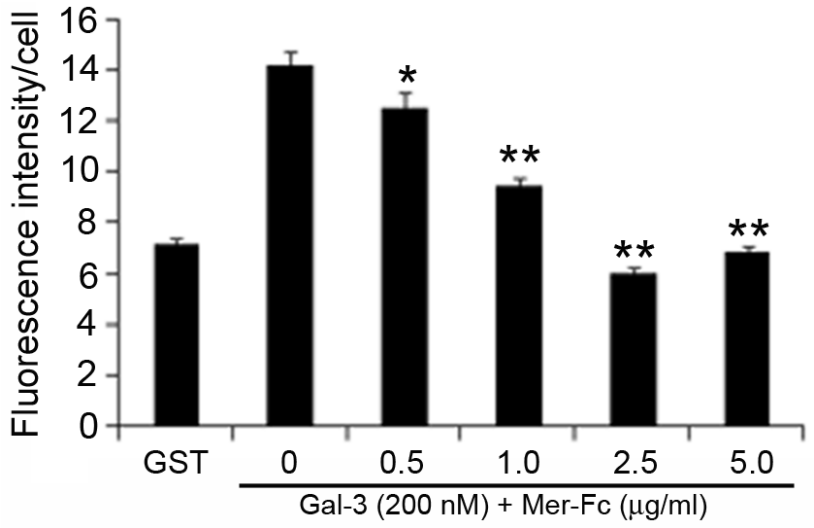
Supplementary Figure 4. **(A)** pHrodo-labeled Jurkat cells are fluorescent only in acidic environment. **(B)** Gal-3 facilitates macrophage phagocytosis of pHrodo-labeled apoptotic Jurkat cells. pHrodo-labeled apoptotic and healthy Jurkat cells were incubated with J774 macrophages and analyzed by confocal microscopy as described in Fig. 3A. The z-stack images of DAPI nuclear signals (blue) and pHrodo signals were superimposed with the cognate bright field images. Bar = 10 μm.



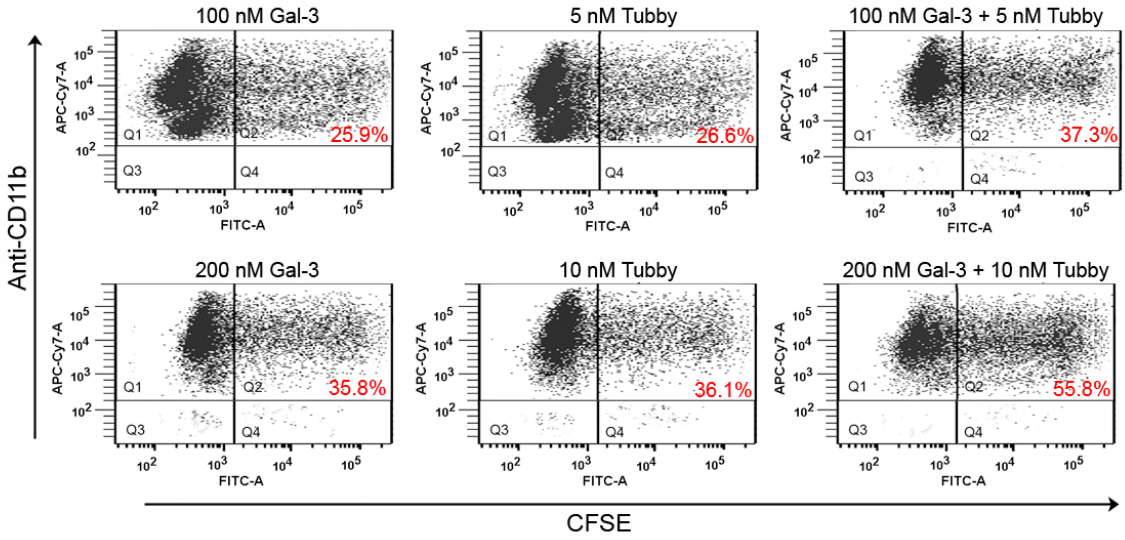
Supplementary Figure 5. Gal-3 does not affect the viability of macrophages. J774 macrophages were incubated with increasing concentrations of GST- Gal-3 for 3 h or 24 h. The viability of the cells were analyzed using trypan blue. (± s.e.m., n = 5)



Supplementary Figure 6. Gal-3 induces MerTK activation in a dose-dependent manner. D407 cells were incubated with indicated concentrations of GST or GST-Gal-3 to induce MerTK activation, as previously described (Caberoy et al., 2010c). MerTK was co-immunoprecipitated using anti-MerTK antibodies and analyzed by Western blot using anti-phospho-MerTK or anti-MerTK antibodies.



Supplementary Figure 7. Gal-3-mediated RPE phagocytosis is blocked by Mer-Fc. CFSE-labeled membrane vesicles were incubated with D407 RPE cells, analyzed for phagocytosis by confocal microscopy, and quantified as described in Fig. 2A and B. The data were analyzed by Tukey-Kramer multiple comparisons test (± s.e.m.; n>100; * $p<0.05$; ** $p<0.001$; versus Gal-3 with no Mer-Fc).



Supplementary Figure 8. Gal-3 and tubby additively facilitate macrophage phagocytosis. Phagocytosis of CFSE-labeled apoptotic Jurkat cells by J774 macrophages was analyzed in the presence of purified Gal-3 or tubby or both with indicated concentrations. The macrophages were treated with trypsin to remove surface-bound Jurkat cells, washed, labeled with APC/Cy7-conjugated anti-CD11b for macrophages and analyzed by flow cytometry, as described in Fig. 3C. The number in each panel indicates the percentage of J774 cells with phagocytosed apoptotic Jurkat cells.