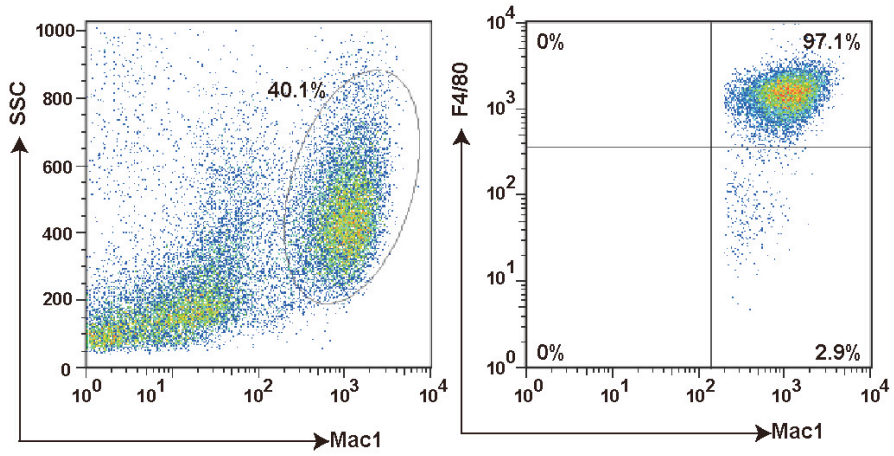
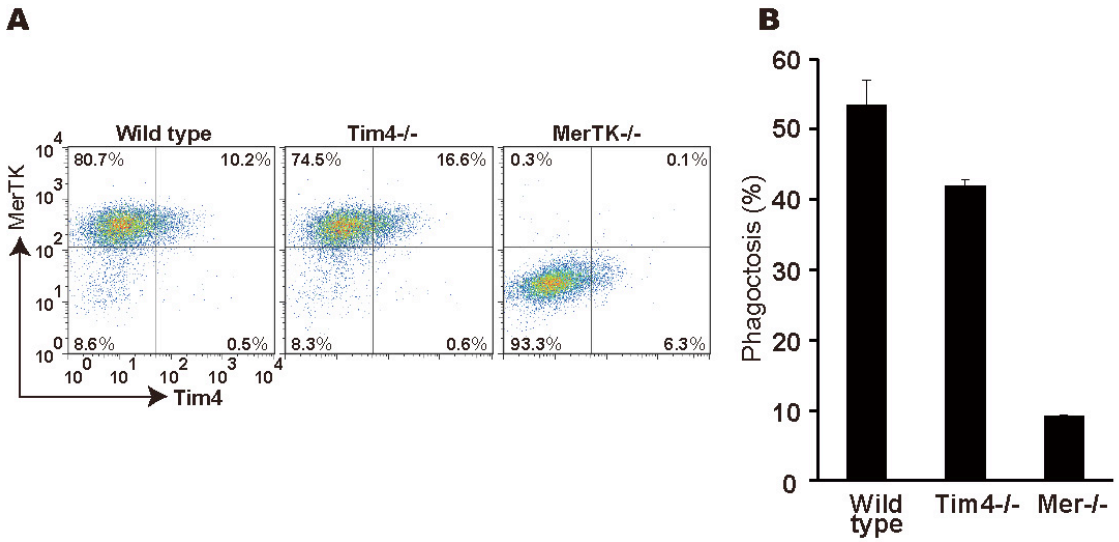
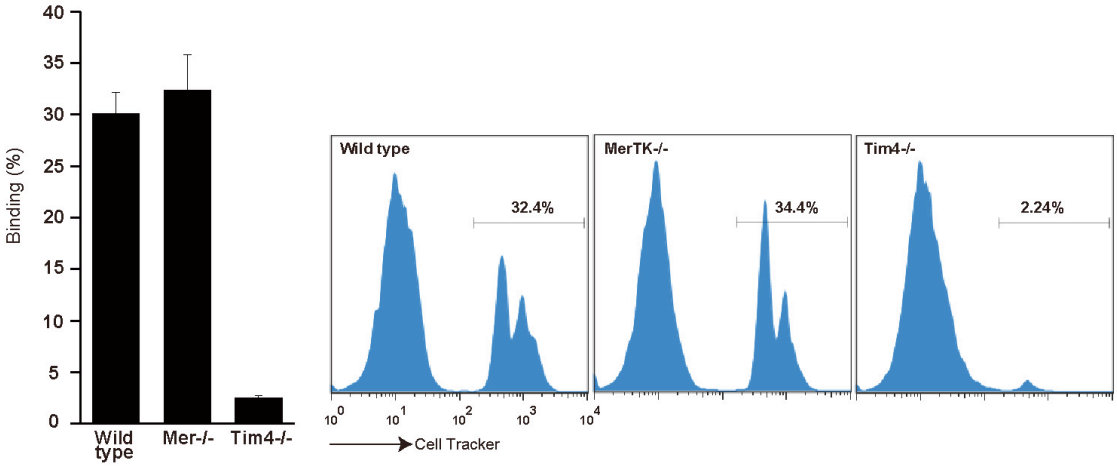


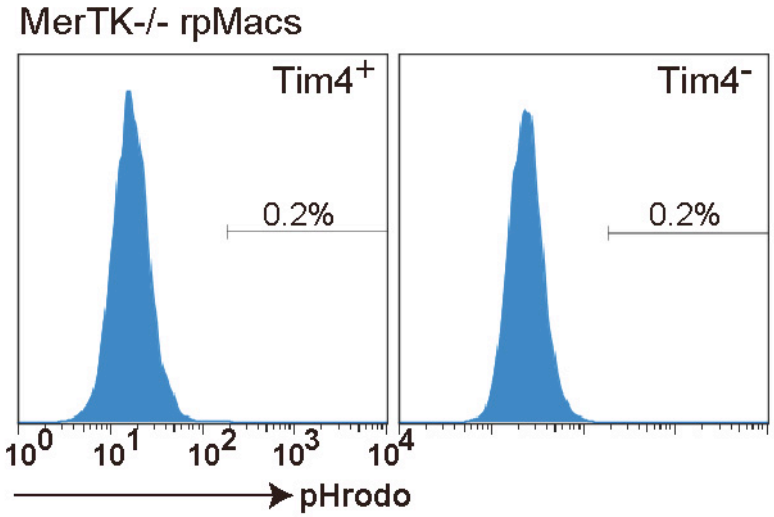
Supplementary Figure 1. Expression of Mac1 and F4/80 in rpMacs. Peritoneal cells from C57BL/6J mice were stained with APC-conjugated anti-mouse Mac1 mAb, and biotinylated anti-F4/80 mAb, followed by staining with Alexa488-conjugated streptavidin, and analyzed by flow cytometry. Left panel shows the staining profile for Mac1. In right panel, the Mac1-positive cells enclosed in left panel are analyzed for Mac1 and F4/80.



Supplementary Figure 2. Requirement of MerTK for the engulfment of apoptotic cells by mouse thioglycollate-elicited peritoneal macrophages. (A) The thioglycollate-elicited peritoneal macrophages (thio-pMacs) were prepared from the wild-type, *Tim4*$^{-/-}$ and *MerTK*$^{-/-}$ mice as described previously (Hanayama et al., 2002), and cultured in DMEM containing 10%FCS. Cells were stained with PerCP-Cy5.5-anti-mouse Mac1, biotinylated goat anti-MerTK, and hamster anti-mouse Tim4, followed by staining with Alexa488-streptavidin and APC-anti-hamster IgG. The stained cells were analyzed by flow cytometry. The expression profiles of MerTK and Tim4 in the Mac1-positive population are shown. (B) The thio-pMacs from wild-type, *Tim4*$^{-/-}$, and *MerTK*$^{-/-}$ mice were incubated with pHrodo-labeled apoptotic thymocytes at 37℃ for 120 min and the percentage of pHrodo-positive cells in Mac1-positive population was determined by flow cytometry. The experiments were performed three times, and the average values are plotted with the S.D. (bars).



Supplementary Figure 3. Thymocytes from 4 week old C57BL/6J mice were labeled with CellTracker Orange at 37°C for 30 min, and incubated with FasL in serum free DMEM at 37°C for 2 h to induce apoptosis. The cells were washed with PBS containing 0.5% BSA and 0.25% globulin, and suspended in PBS containing 0.5% BSA. Peritoneal cells from wild-type, *Tim4*$^{-/-}$ and *Mer*$^{-/-}$ mice were incubated with CellTracker-labeled cells at 37°C for 30 min, and analyzed by flow cytometry for the CellTracker-positive cells in Mac1-positive population. The number indicates the percentage of CellTracker-positive cells. The experiments were done in triplicate, and the average values with S.D. (bars) are plotted. At right, a representative CellTracker-staining profile of Mac1-positive cells is shown.



Supplementary Figure 4. No engulfment of apoptoti cells by MerTK$^{-/-}$ rpMacs. The rpMacs (1 x 10^5 cells) from the MerTK$^{-/-}$ mice were incubated at 37°C for 60 min with pHrodo-labeled apoptotic thymocytes (1 x 10^6 cells). The cells were stained with PerCP-Cy5-anti mouse Mac1, hamster anti-Tim4, followed by staining with APC-antihamster IgG, and analyzed by flow cytometry. The FACS profiles for pHrodo-positive cells in each Tim4+ and Tim4- population of Mac1-positive cells are shown. The number indicates the percentage of pHrodo-positive cells in each population.