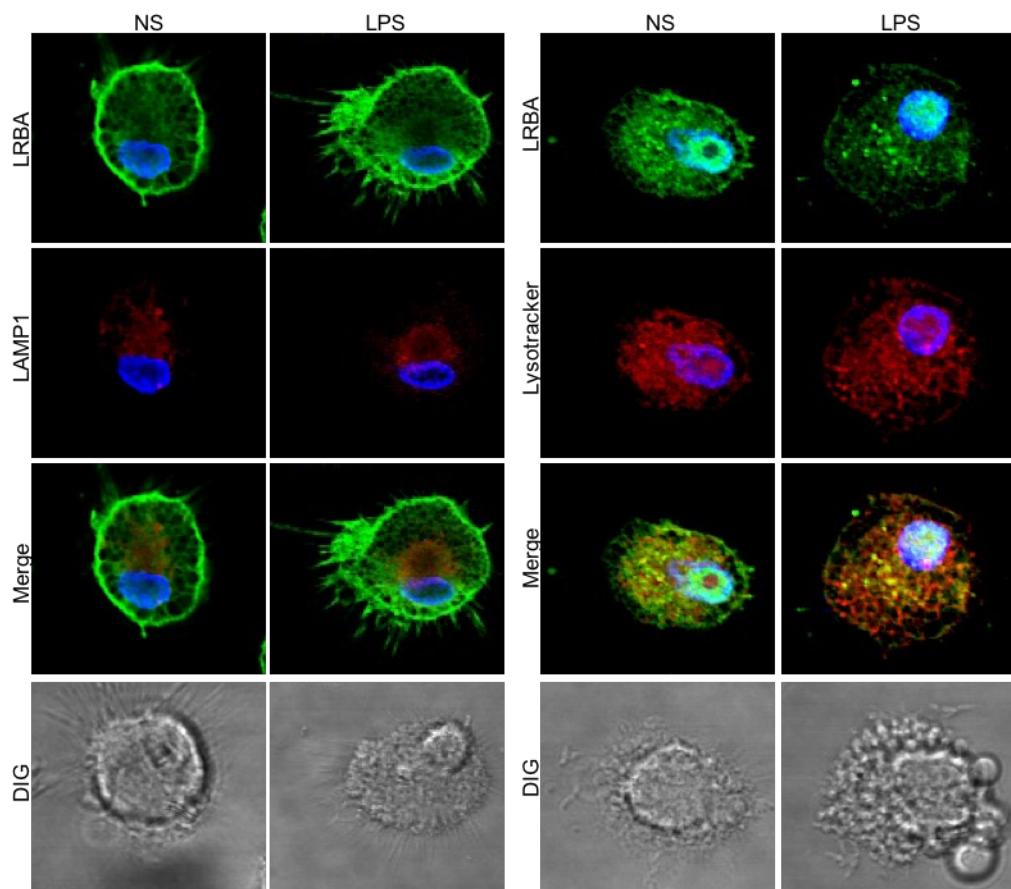
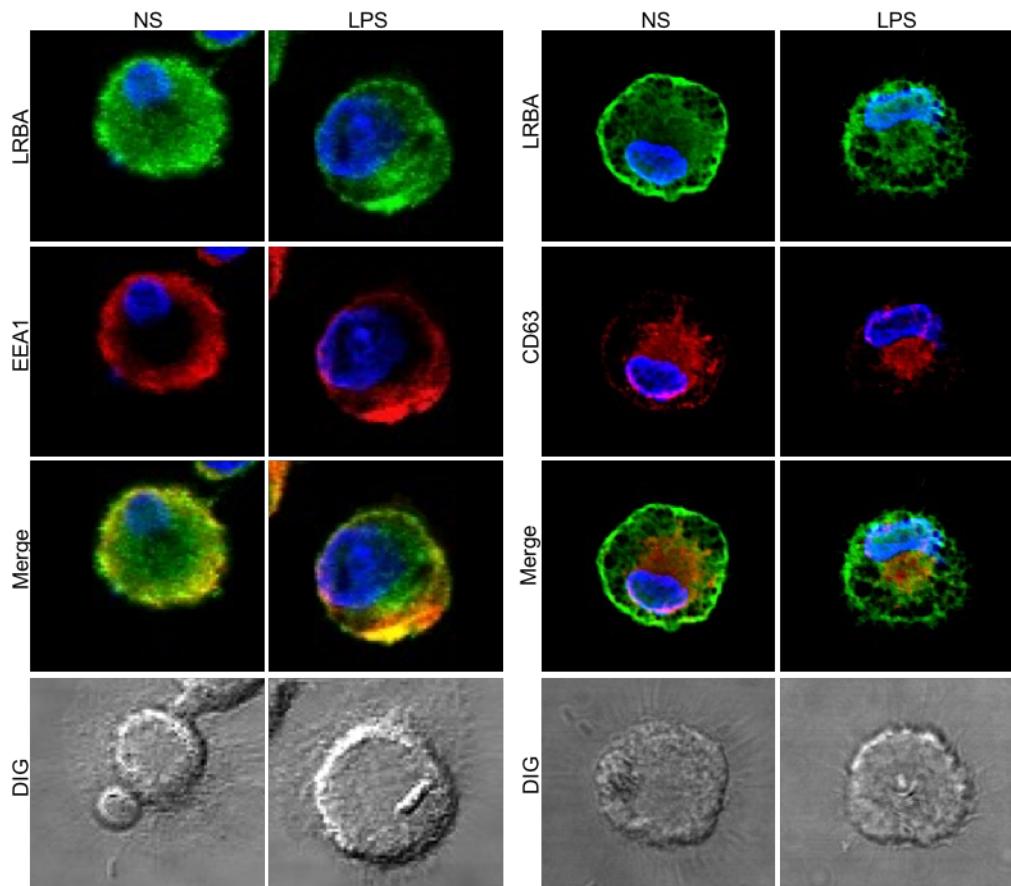
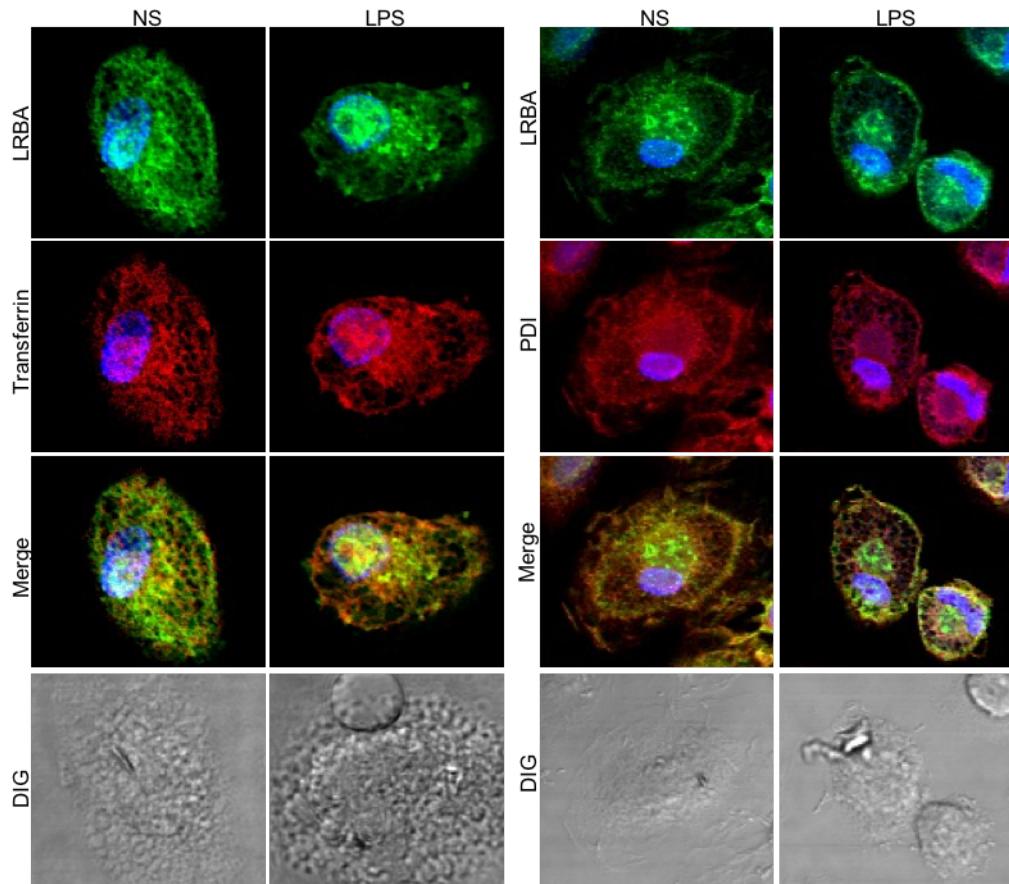
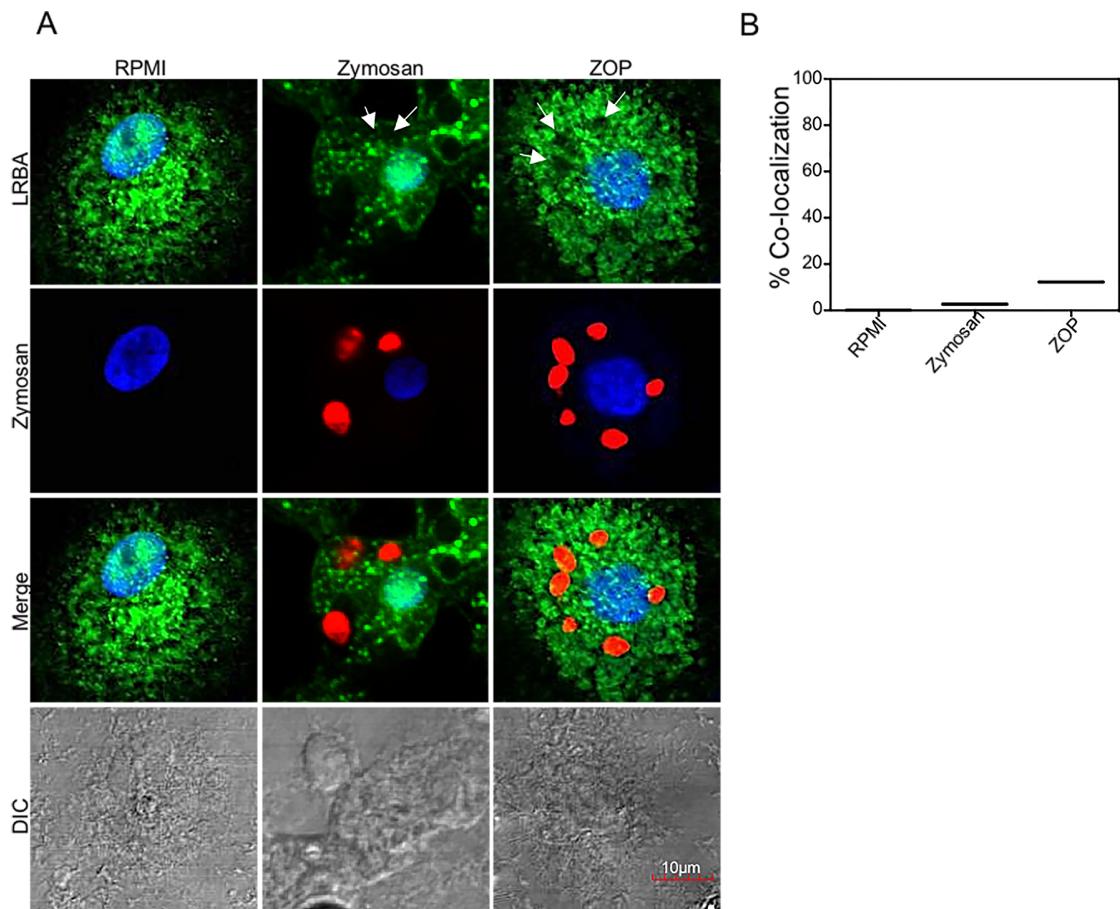


Supplementary Figure 1: Differentiation of human mononuclear phagocytes (MP) and LRBA expression in these cells. Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors and monocytes were obtained after 4h cell adherence in medium supplemented with 0.5% autologous serum. After washing twice with PBS, adherent cells were incubated 5 days in medium supplemented with 10% autologous serum to obtain MP. Shown is the expression of CD20 and CD14 by FACS in cultures before and after adherence to evaluate the percentages of monocytes and B cells, respectively (A). Also, expression of CD86 and HLA-DR (B) and cell morphology (C) was analyzed in the MP monolayer to evaluate purity before and after differentiation. After stimulation with LPS (1 μ g/mL) at different timepoints, the production of TNF-alpha was evaluated by ELISA (D) or FACS w/o different stimuli (F). Considering that anti-LRBA antibodies obtained commercially are polyclonal, the specificity of recognition was also evaluated using the LRBA peptide SVLMVSKYRDILEPQNERHSQSCTETGSELENVSLSEITPAAFSTLTTASVEESES TSSARRRDSGIGEETATGLGSHVEVTPTAPPGVSAAGPDAISEVLSTLSLEVNKS PETKNDRGNDLDTKATPSVSV as a competitive reagent at different concentrations. The percentage of the recognition of the cell LRBA protein was calculated (G). TNF: Tumor necrosis factor, IL: Interleukin, NS: Non-stimulated, LPS: Lipopolysaccharide, IFN: Interferon, Ab: Antibody. Magnification: 60X.

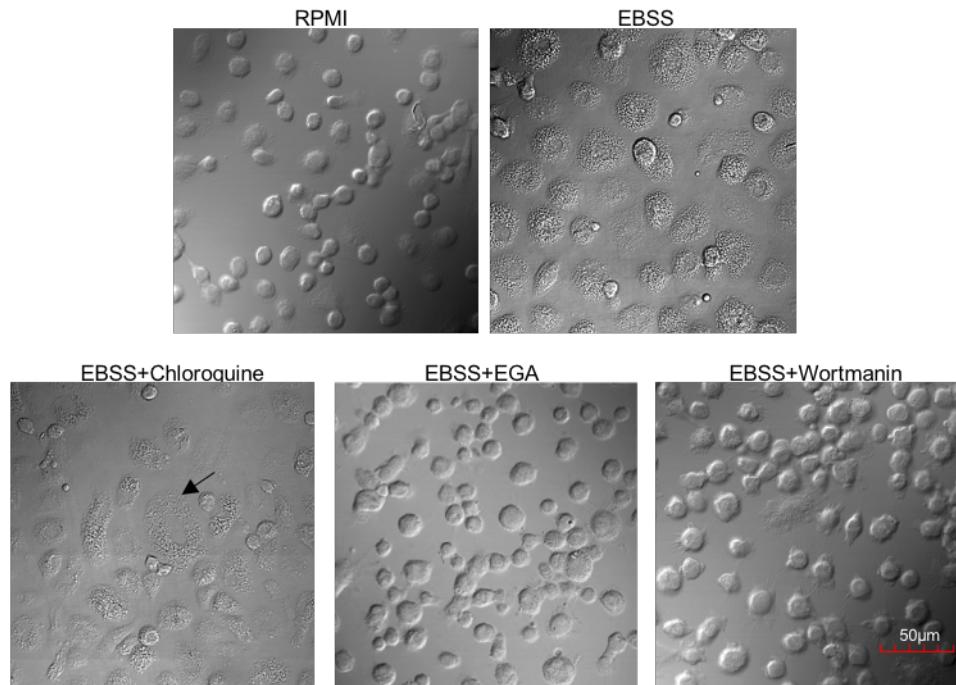




Supplementary Figure 2. Co-localization of LRBA with vesicular markers in mononuclear phagocytes (MP). MP from healthy donors were obtained. LRBA and the different vesicular markers were intracellularly stained on cover glasses and co-localization was visualized by confocal microscopy and analyzed using Pearson correlation. A representative example of the co-localization from LRBA (green) with EEA1, CD63, LAMP1, Lysotracker, Transferrin or PDI (red) in MP w/o LPS is shown. Magnification: 63X. Lyso: Lysotracker, Transf: Transferrin NS: Unstimulated cells, LPS: Lypopolissacharide. Magnification: 63X



Supplementary Figure 3. Role of LRBA in phagocytosis. Mononuclear phagocytes (MP) from a healthy donor were exposed one hour to fluorescent-labeled zymosan particles w/o 30 min of previous opsonization with a pool of human sera. Shown is the co-localization of intracellular LRBA (green) and the labeled zymosan (red) (A) and the Pearson correlation analysis (B). Results are representative from three experiments with MP obtained from different healthy controls (n=3). The white arrows indicate that LRBA was excluded from the zymosan phagosomes. p value = 0.977. Magnification: 63X.



Supplementary Figure 4. Morphological changes in MP after exposure to the autophagy flux and its inhibition. MP cells from healthy donors were incubated in medium alone (RPMI) or exposed to the autophagy inductor EBSS alone or together with autophagy inhibitors chloroquine (30 μ g/mL), EGA (1mg/mL) and wortmannin (1mg/mL) for 1 h. ~~Shown is the change cellular morphology.~~ The white arrows indicate the morphology of small cells with few vacuoles. On the contrary, the black arrows indicate big cells with many and big vacuoles. Results are representative from four experiments with MP obtained from different healthy controls (n=4). Magnification: 63X