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Supplemental Data

Extracellular Histones Inhibit Efferocytosis

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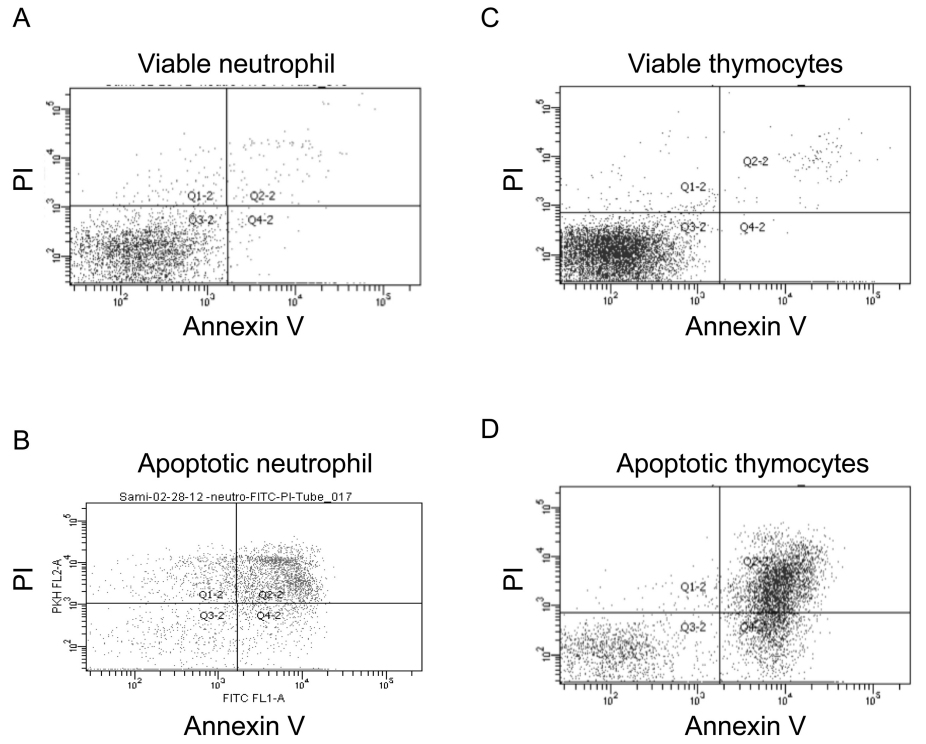
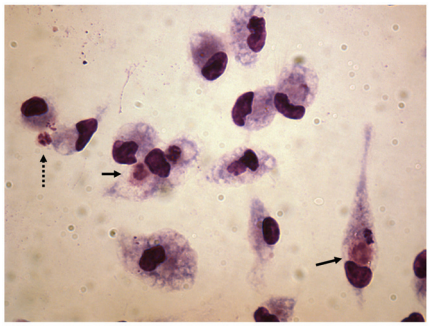
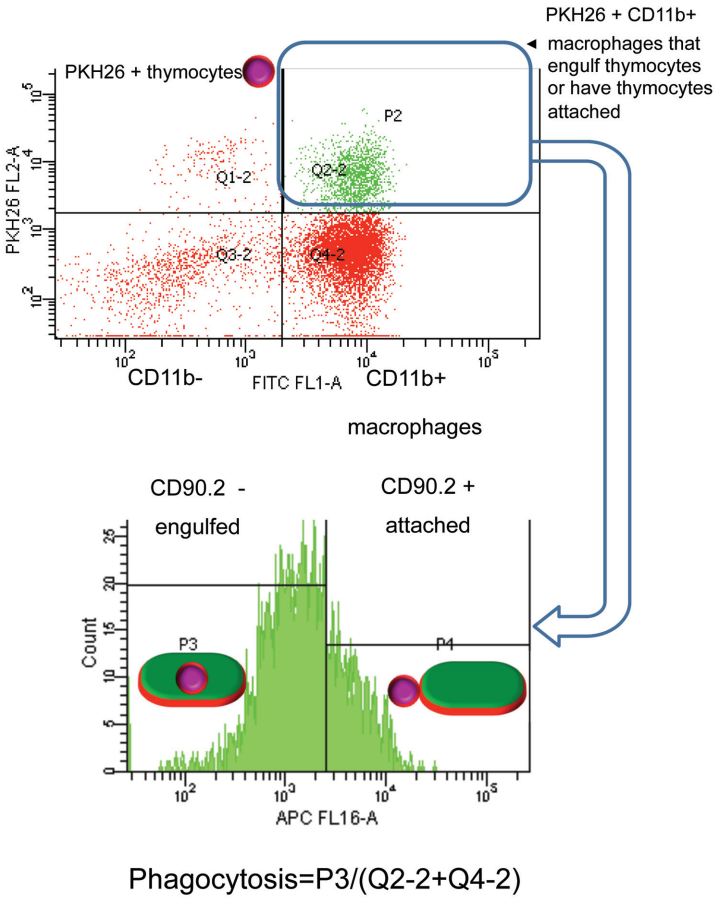


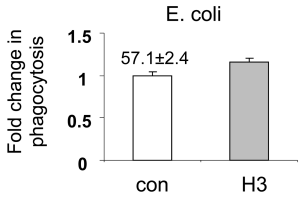
Supplementary Figure S1. Representative flow cytometry analyses of viable and apoptotic neutrophils (A-B) and thymocytes (C-D). Viable and apoptotic neutrophils and thymocytes were stained with Annexin V and PI. As shown in B and D, percentage of apoptotic neutrophils or thymocytes is approximately 80% .



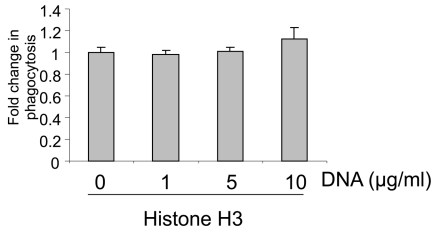
Supplementary Figure S2. A representative image of phagocytosis assays using apoptotic neutrophils as targets and macrophages as phagocytes. Dashed arrow points to a neutrophil that is attached to a macrophage. Solid arrows point to neutrophils that are engulfed by macrophages.



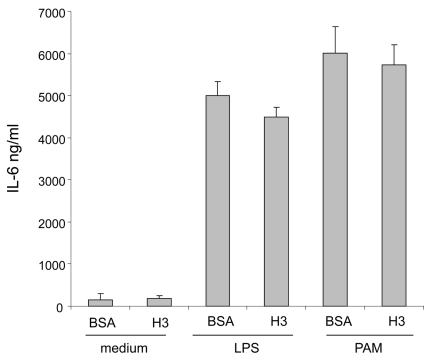
Supplementary Figure S3. A representative diagram of phagocytosis assays using apoptotic thymocytes as targets and macrophages as phagocytes. After incubation of apoptotic thymocytes with macrophage monolayers, non-ingested cells were removed by washing three times with ice-cold PBS. Cells were collected in PBS containing 1% albumin, FITC conjugated CD11b (macrophage marker) antibody and APC conjugated CD90.2 (thymocyte marker) antibody. Flow cytometry was performed. The phagocytic index was calculated as the ratio of FITC+PKH26+APC- cells to all macrophages gated. Engulfed thymocytes are not accessible to the APC conjugated CD 90.2 antibody. Therefore, FITC+PKH26+APC-cells are macrophages that have engulfed PKH labeled thymocytes, whereas the APC+PKH26+FITC+ cells were macrophages to which thymocytes are adherent, but not engulfed. Free apoptotic thymocytes stained without or with APC conjugated CD90.2 to set the threshold for CD90.2 positive and negative populations.



Supplementary Figure S4. Phagocytosis of *E. coli* is not inhibited by extracellular histone H3. FTIC labeled heat inactivated *E. coli* were resuspended in 300 µl medium containing 10 µg BSA or histone H3 and then added to macrophage monolayers. 20 min after the phagocytosis, cells were washed thoroughly with PBS and collected for flow cytometry analysis. The percentage of FITC+ macrophages for the BSA group was set as 1. n=3, mean±SD.



Supplementary Figure S5. Genomic DNA does not affect the inhibitory activity of histone H3 on phagocytosis of apoptotic thymocytes. Apoptotic thymocytes were resuspended in 300 µl medium containing 10 µg/ml BSA histone H3 and 0, 1, 5, 10 µg/ml calf thymus genomic DNA. Cells were added to macrophage monolayers and efferocytosis assays performed. n=3, mean±SD.



Supplementary Figure S6. Histone H3 does not activate macrophages or affect inflammatory response of macrophages to TLR2 or TLR4 activation. Macrophages were treated with 10 µg/ml BSA or histone H3, or 10ng/ml LPS or 1 µg/ml PamCSK3 in the presence of 1 µg/ml BSA or histone H3 for 24 h. IL-6 levels in the culture supernatants were determined by ELISA.