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 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.