Supplementary Figure Legends

Supplementary Figure 1 TLR9 is cleaved into two distinct fragments by cathepsins as determined by mass spectrometry. (**a**) Magnified view of the silver stained gel in **Fig. 1c.** Asterisks denote the 65 and 45 kDa bands that were identified as TLR9 polypeptides by LC/MS/MS. (**b**) LC/MS/MS analysis of the 45 kDa band revealed exclusively peptides matching the N-terminal part of TLR9 (top), whereas the 65 kDa band only revealed peptides matching the C-terminal part of TLR9 (bottom). Peptide coverage is shown as yellow bars and peptides recovered are highlighted in the TLR9 protein sequence in red.

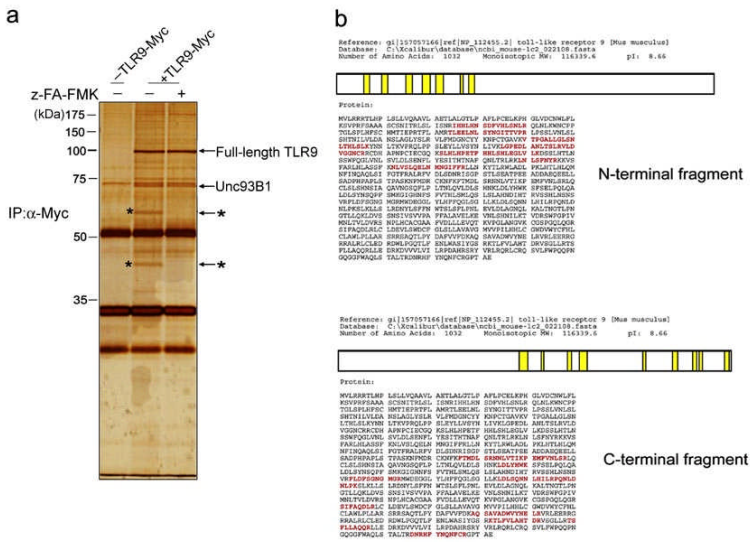
Supplementary Figure 2 Cleavage of TLR9 is a late biosynthesis event. RAW macrophages stably expressing TLR9-Myc were metabolically labeled for 1.5 h and cells were lysed at indicated chase times. Lysates were subjected to immunoprecipitation with anti-Myc, followed by dissociation of the immune complex and reimmunoprecipitation with anti-Myc. Samples were digested with EndoF where indicated and visualized by SDS-PAGE.

Supplementary Figure 3 TLR7 is not cleaved by cathepsins. (**a**) RAW macrophages stably expressing TLR9-Myc were treated with DMSO or z-FA-FMK, were metabolically labeled, and were lysed. Anti-Myc immunoprecipitates were digested with EndoH or EndoF where indicated and visualized by SDS-PAGE. Arrows indicate

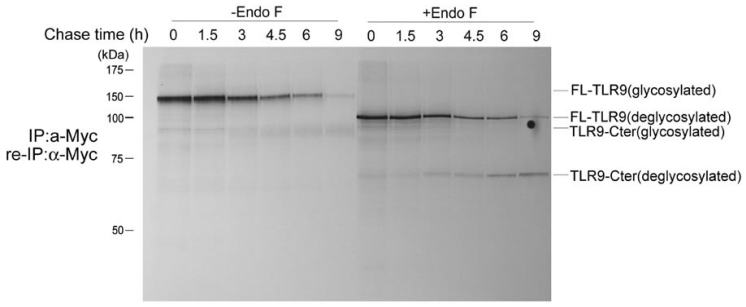
the C- and N-terminal fragment of TLR9-Myc (**b)** Endogenous TLR7 was immunoprecipitated with anti-TLR7 from metabolically labeled RAW macrophages treated with either DMSO or z-FA-FMK. Immunoprecipitates were digested with EndoH or EndoF where indicated and visualized by SDS-PAGE.

Supplementary Figure 4 Model of TLR9 cleavage in the endolysosomal compartment by lysosomal proteases. After cleavage by lysosomal proteases including cathepsins L and S, the C-terminal fragment of TLR9, consisting of leucine rich repeats 15-26, the transmembrane domain and the cytoplasmic C-terminus essential for interaction with adaptor molecules, binds CpG DNA and initiates signaling.

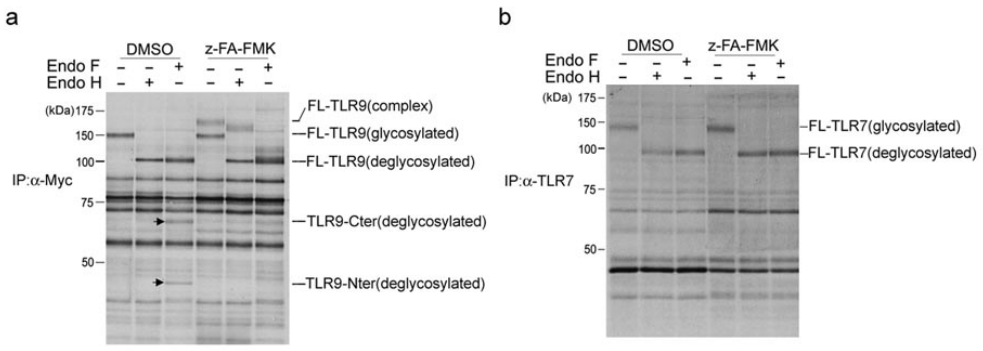
Supplementary Fig. 1.Park et al.



Supplementary Fig. 2. Park et al.



Supplementary Fig.3. Park et al.



Supplementary Fig.4. Park et al.