

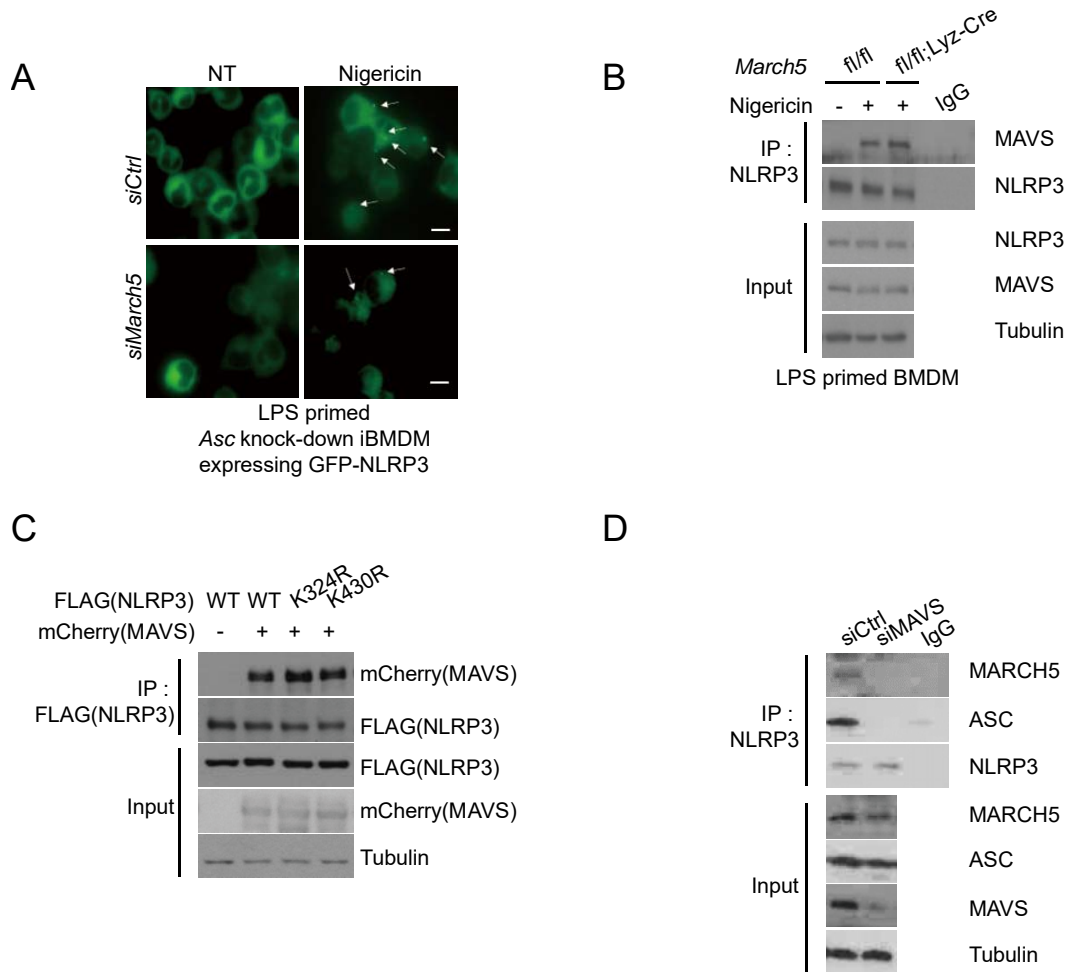
MARCH5-dependent NLRP3 ubiquitination is required for mitochondrial NLRP3-NEK7 complex formation and NLRP3 inflammasome activation

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Appendix

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Appendix Figure S1. MAVS is essential for NLRP3-ASC-MARCH5 complex formation.

(A) Fluorescence microscopy images of NLRP3 oligomerization. *Asc* was knocked down in immortalized BMM and cells expressing GFP-NLRP3 as well as *siControl* or *siMarch5*. Cells were stimulated with 200 ng/ml LPS for 4 hr and 15 μ M nigericin for 30 min. White arrows indicate NLRP3 oligomerization. Bars, 10 μ m.

(B) *March5^{fl/fl}* and *March5^{fl/fl;Lyz-Cre}* BMDMs were stimulated with 200 ng/ml LPS for 4 hr and 5 mM ATP for 30 min.

(C) HEK293T cells were cotransfected with mCherry-MAVS and WT or mutant NLRP3. Cells were treated with 200 ng/ml LPS

and 15 μ M nigericin. Cell lysates were immunoprecipitated with FLAG-M2 beads. The proteins in the precipitate were analyzed by immunoblotting with the indicated antibodies.

(D) THP-1 cells were transfected with siControl and siMAVS. After stimulation with 200 ng/ml LPS for 4 hr and 5 mM ATP for 30 min, cell lysates were immunoprecipitated with NLRP3 antibody. The indicated proteins were detected with the corresponding antibodies.