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Supplementary Materials for

The interaction between IKKα and LC3 promotes type I interferon production through the TLR9-containing LAPosome

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This PDF file includes:

Supplementary Data

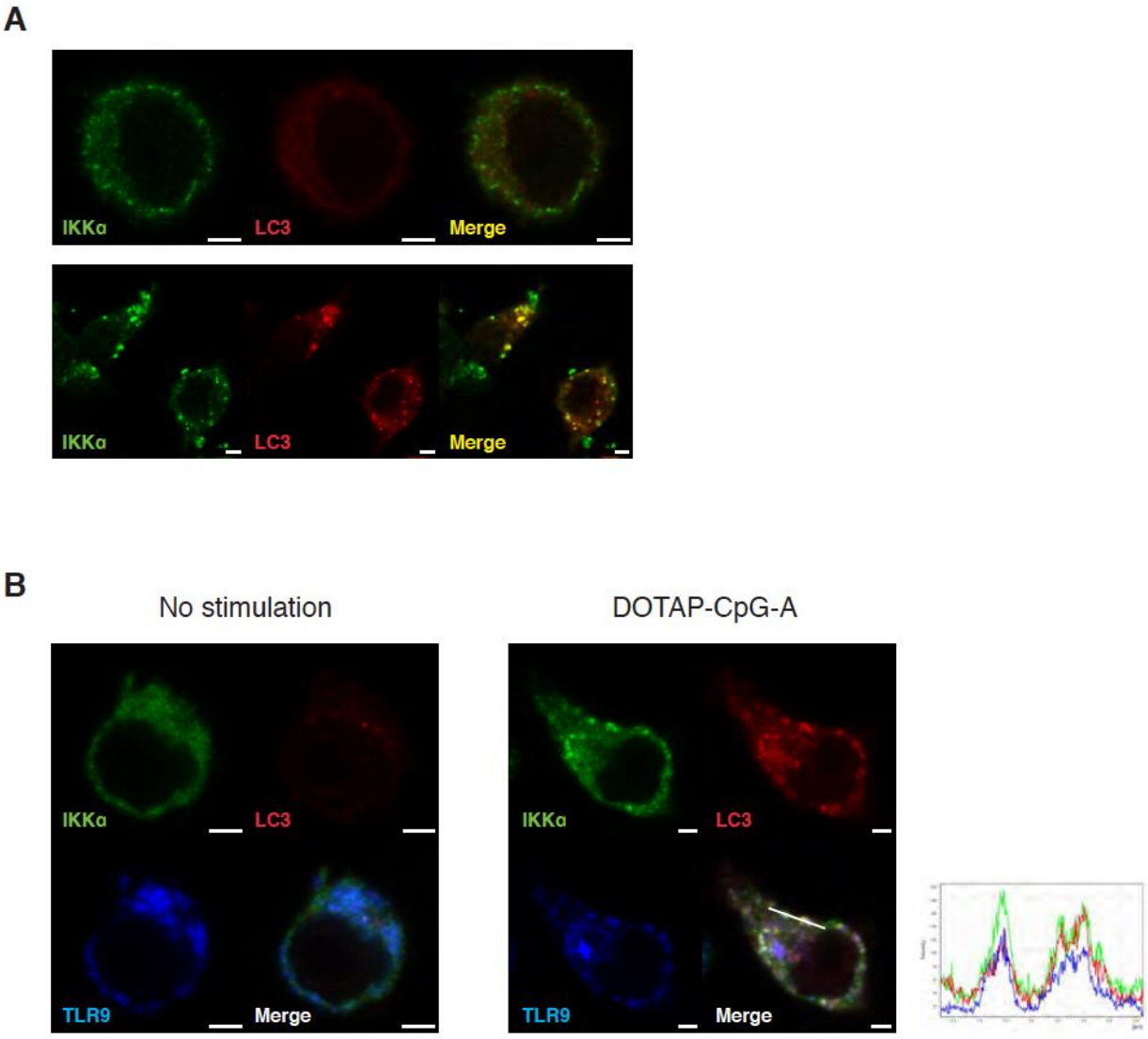

Fig. S1. LC3 binds to TLR9 and IKKα after DOTAP–CpG-A stimulation.

(A) RAW264.7 cells stably expressing Cherry-LC3 were stimulated with DOTAP-CpG-A for 6 hours before staining and analysis by confocal microscopy. Images are representative of 3 independent experiments. Scale bars: 5um (B) RAW264.7 cells stably expressing Cherry-LC3 and TLR9-HA were stimulated with DOTAP-CpG-A for 6 hours before staining and analysis by confocal microscopy. Images are representative of 3 independent experiments. Scale bars: 5um

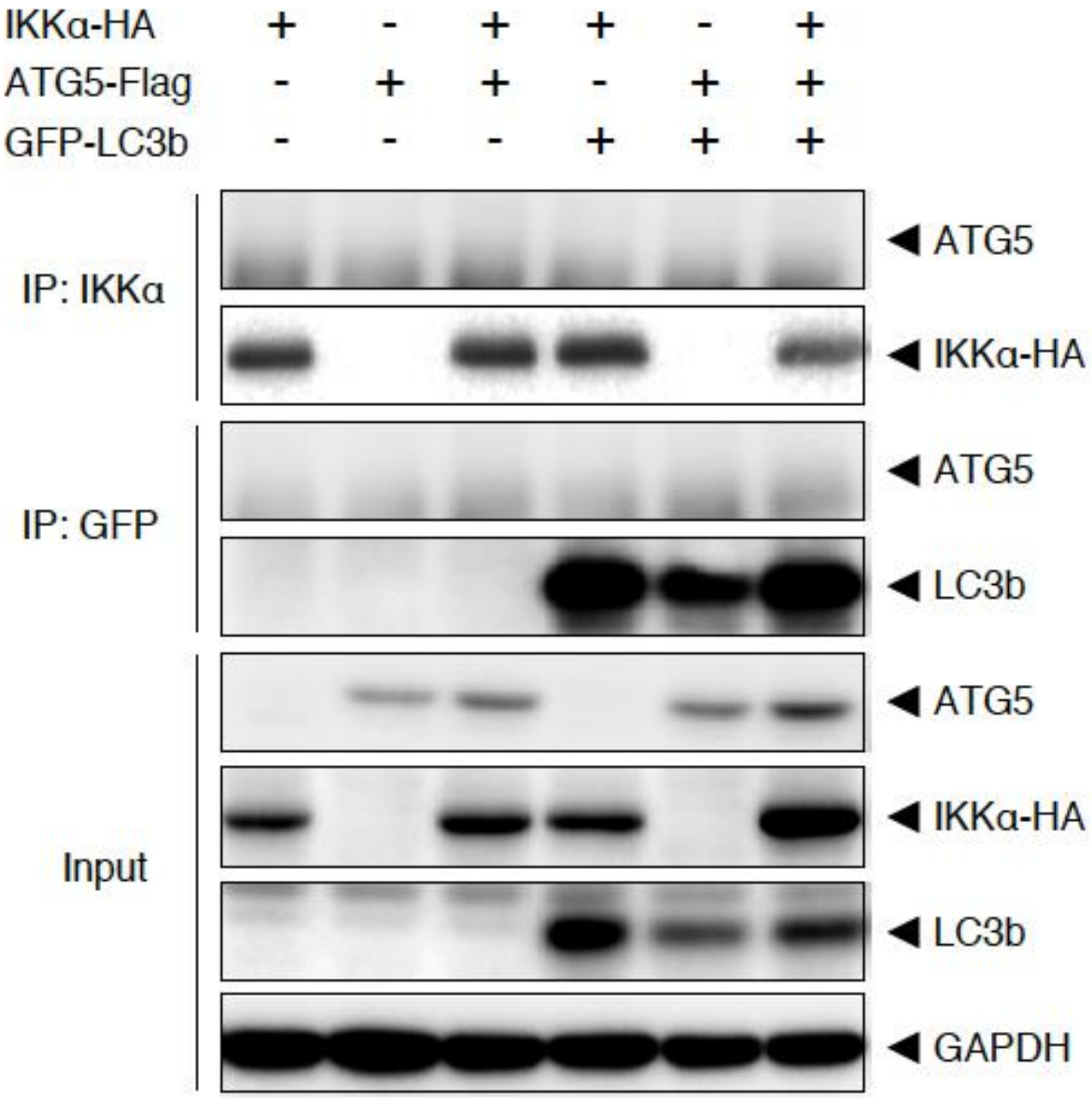

Fig. S2. ATG5 does not interact with LC3 or IKKα.

HEK293T cells were transfected with wild-type IKKα-HA, GFP-LC3b, and ATG5-Flag plasmids. Cell lysates were immunoprecipitated for IKKα or GFP and blotted for the indicated proteins. Blots are representative of 3 independent experiments.

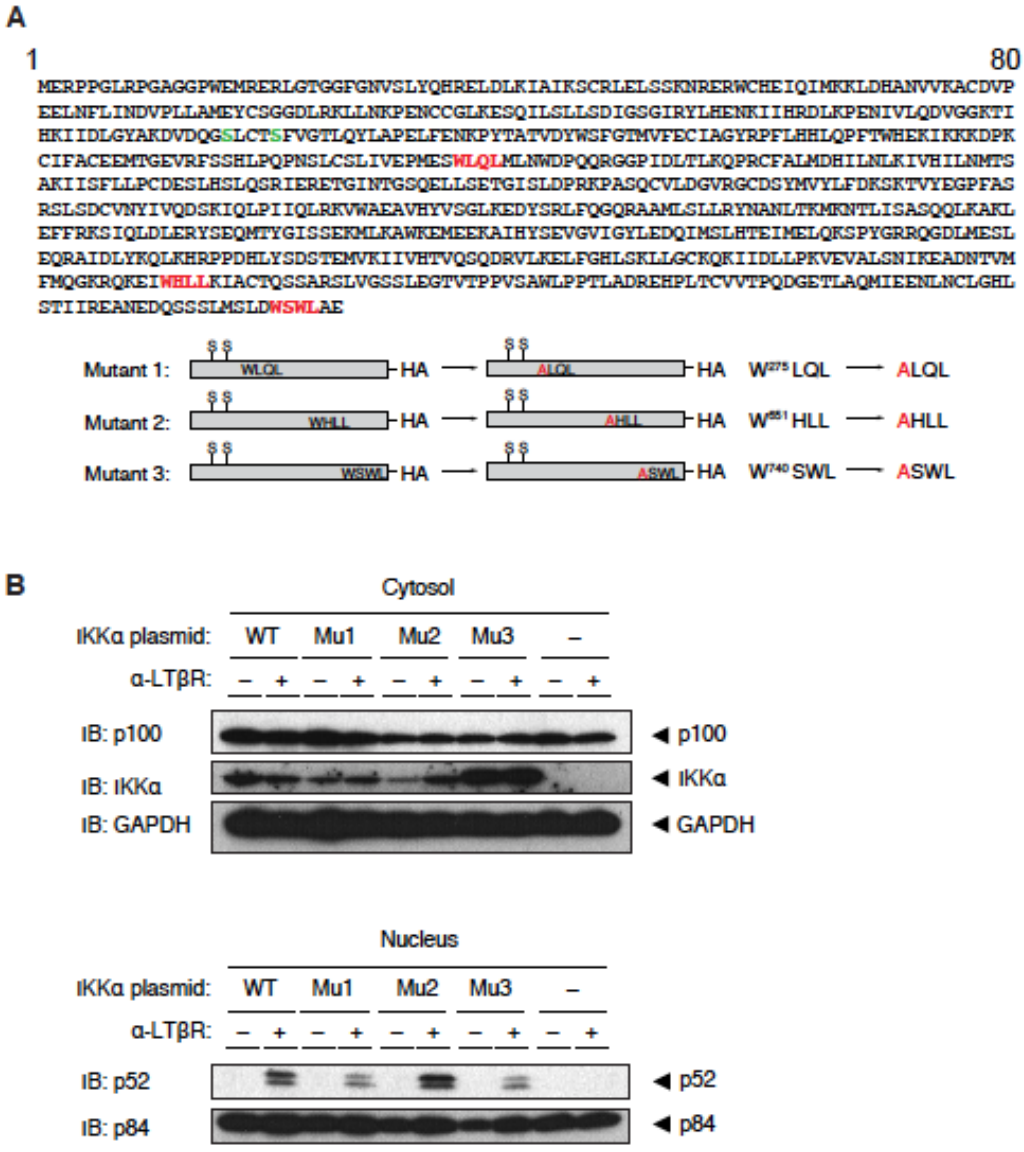

Fig. S3. LIR mutants of IKKα maintain intact kinase activity.

(A) IKKα sequence showing two serine residues in green phosphorylated upon activation of IKKα, and three LIRs in red. (B) *Ikkα*$^{-/-}$ mouse embryonic fibroblasts (MEFs) were retrovirally reconstituted with different IKKα constructs (wild-type or LIR mutants) and stimulated with agonistic antibody against LTβR (300ng/ml) for 17 hours. Cytoplasmic and nuclear extracts were prepared and analyzed by Western blotting with antibodies against the indicated proteins. Blots are representative of 3 independent experiments.

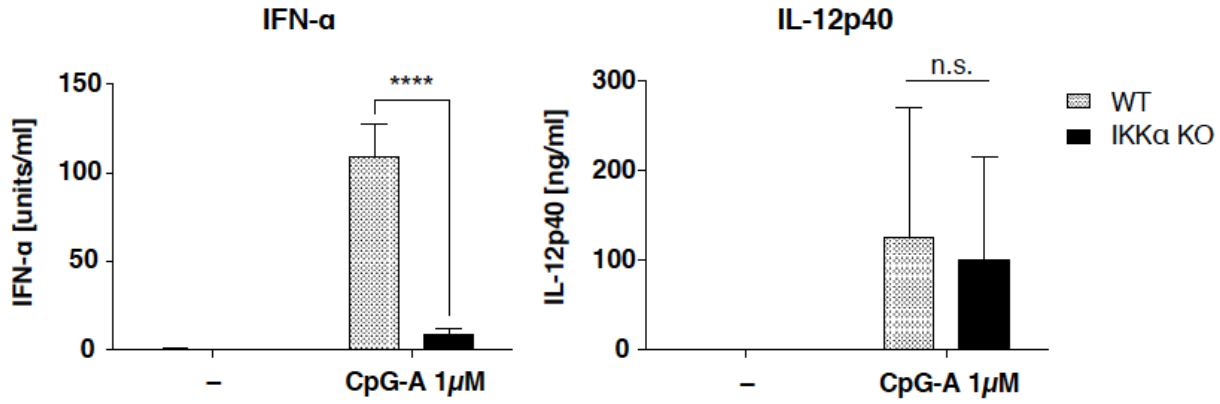

Fig. S4. Fms-related tyrosine kinase 3 ligand–derived Ikkα-deficient pDCs show impaired IFN-α production but intact IL-12p40 after CpG-A stimulation.

Bone marrow derived pDC from *Ikkα*$^{-/-}$ were stimulated with 1μM CpG-A and the cytokine accumulation in the supernatant 24 hours later was analyzed by ELISA. Data are means ± SEM from 3 independent experiments. **, *** denote $P < 0.01$, $P < 0.001$, respectively.

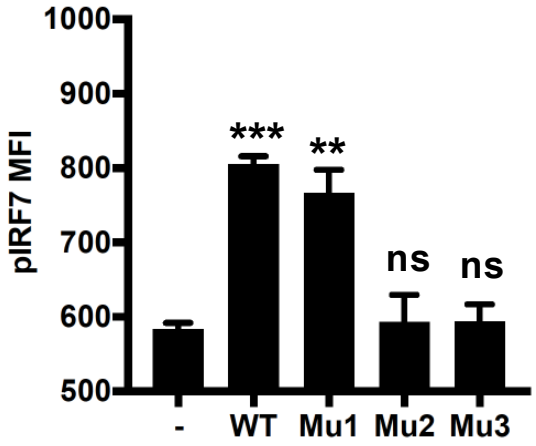

Fig. S5. WT and LIR-1 but not LIR-2 or LIR-3 mutant IKKα induces IRF7 phosphorylation.

HEK293T cells were transfected with IKKα-HA (wild-type or LIR-1/LIR-2/LIR-3 mutants) and human IRF7, Myd88, TRAF3, TRAF6, GFP-LC3b. After 48 hours, cells were fixed and permeabilized to detect intracellular phosphorylated IRF7 expression by flow cytometry. Data are means ± SEM from 3 independent experiments.

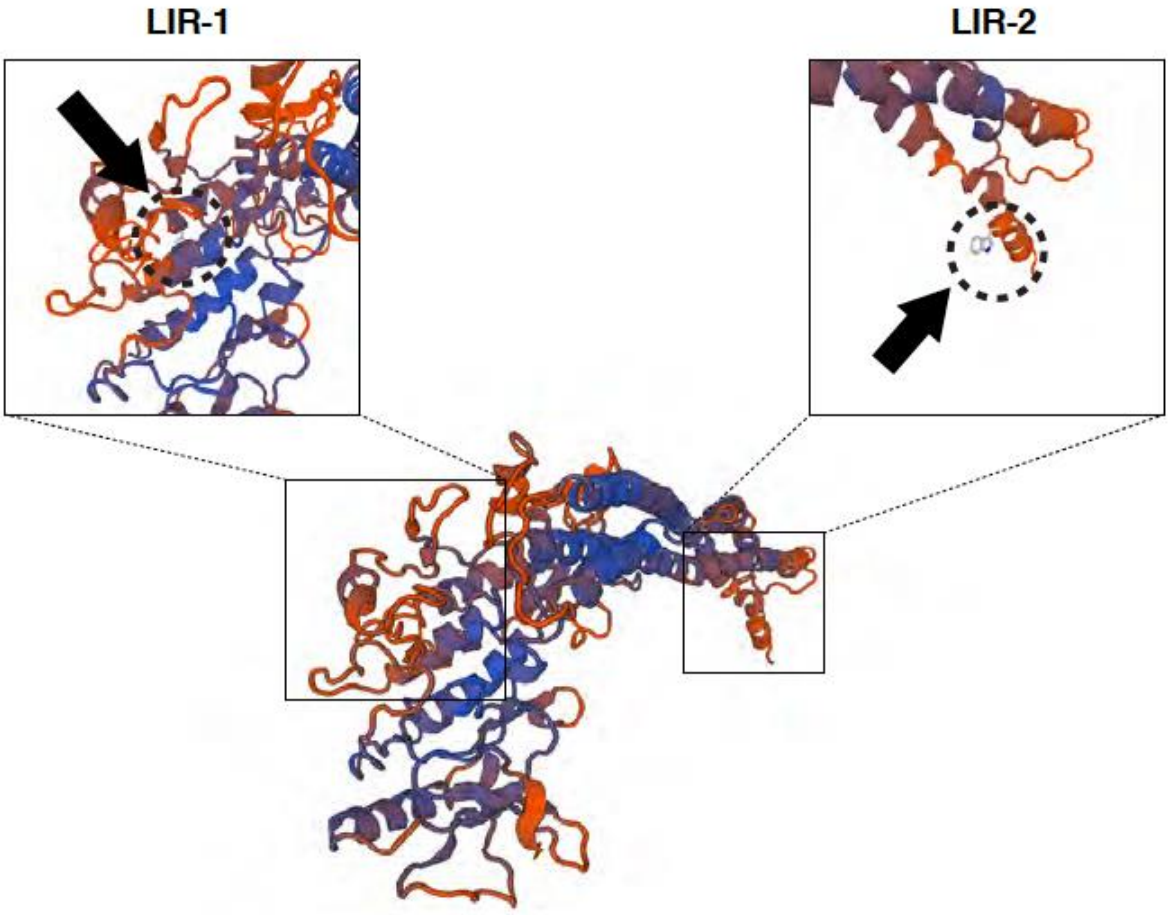

Fig. S6. LIR prediction based on IKKβ structure by SWISS-MODEL.

Two LIRs (LIR-1, LIR-2) of IKKα are shown by black arrows. The zoomed-in images are shown in boxes. LIR-2 and LIR-3 (not shown) are on the C'-terminus region and are accessible, while LIR-1 is buried within the protein near the kinase core.