

Supplementary Materials for
**The interaction between IKK α and LC3 promotes type I interferon
production through the TLR9-containing LAPosome**

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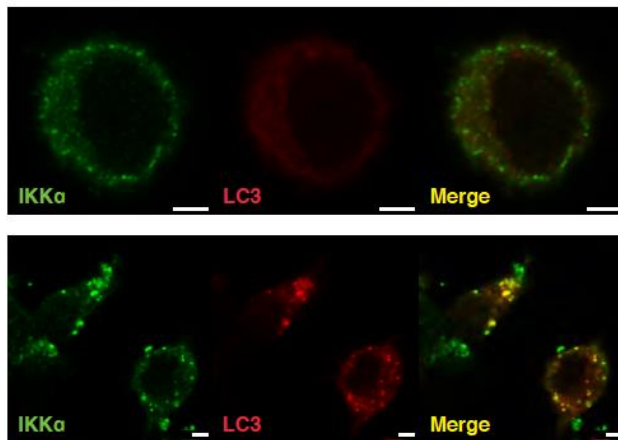
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Supplementary Data

A



B

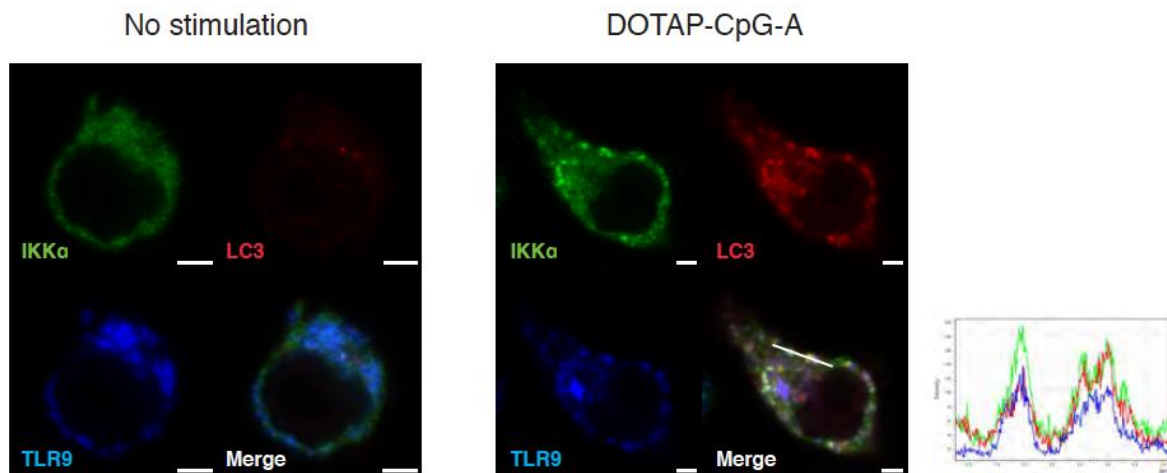


Fig. S1. LC3 binds to TLR9 and IKK α after DOTAP-CpG-A stimulation.

(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: 5 μ m (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: 5 μ m

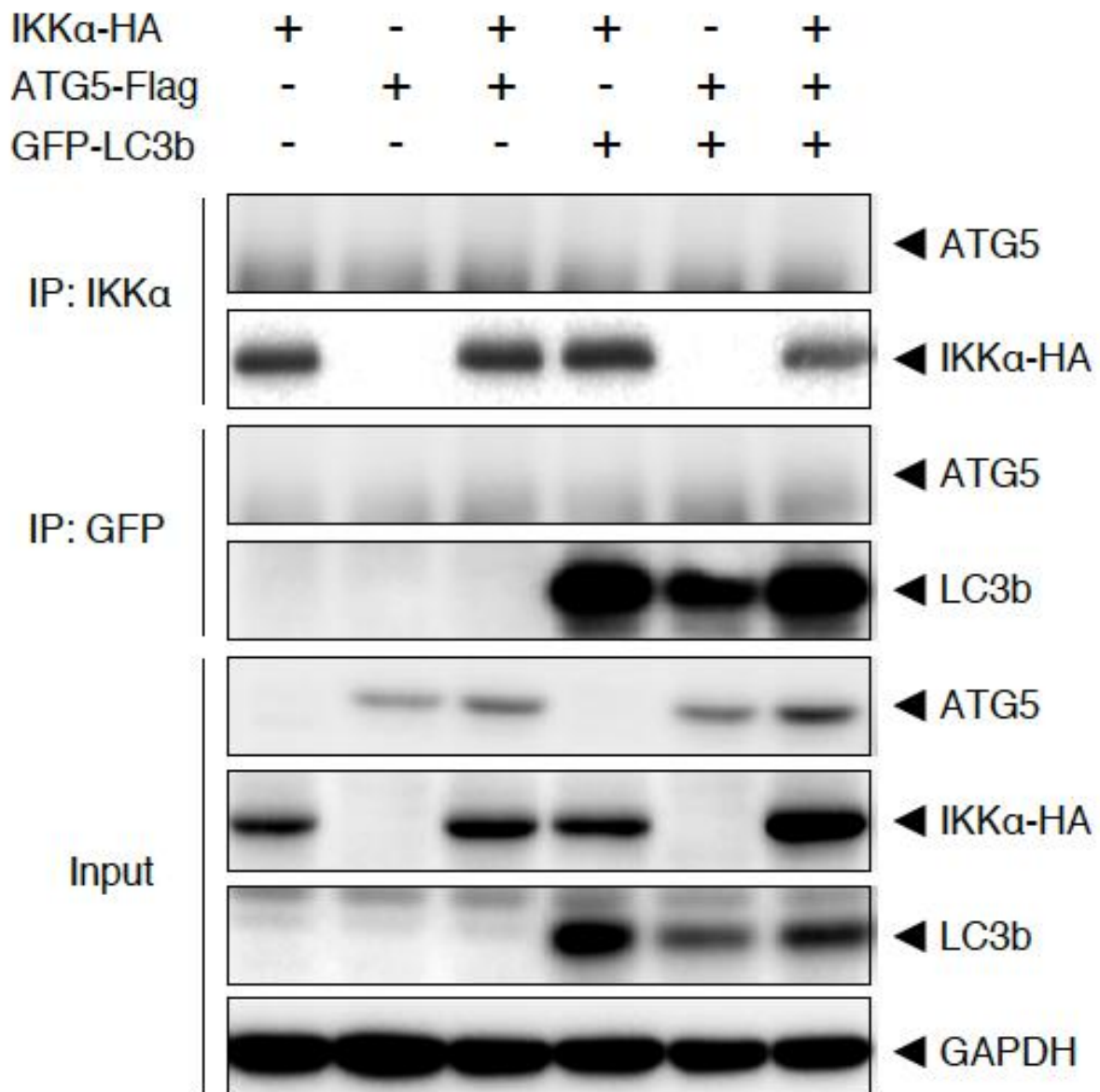


Fig. S2. ATG5 does not interact with LC3 or IKK α .

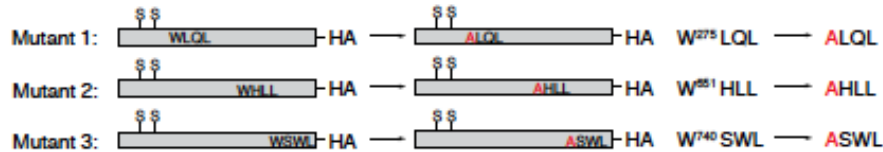
HEK293T cells were transfected with wild-type IKK α -HA, GFP-LC3b, and ATG5-Flag plasmids. Cell lysates were immunoprecipitated for IKK α or GFP and blotted for the indicated proteins. Blots are representative of 3 independent experiments.

A

1

80

MERPPGLRPGAGGPEWEMRERLGTGGFGNVSLYQHRELDLKIARKSRIELSSKNRERWCHEIQIMKKLDHANVVKACDVP
 EELNPLINDVPLLAMEYCSGGDLRKLKLNKPENCCGLKESQILSLLSDIGSGIRYLHENKI IHRDLKPENIVLQDVGGKTI
 HKIIDLGYAKDVDQGS^SLC^STFVGTLLQYLAPELFENKPYTATVDYWSFGTMVFECIAGYRPFLLHHLQPF^TWHEKIKKKDPK
 CIFACEEMTGEVRFSSHLQPNSGLCSLIVEPMES^WL^QLMLNWDPPQORGGPIDLTLKQPRCFALMDHILNLKIVHILNMTS
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 EQRAIDLRYQLKRRPPDHLYSDESTEMVKIIVHTVQSQDRVLKELFGHLSKLLGCKQRKIIDLLPKVEVALSNIREADNTVM
 FMQGRQKEI^WH^LL^LKIACTQSSARSLVSSLEGTVTPVPSAWLPP^TLADREHPLTCVVTPQDGETLAQMIEENLNLGHL
 STIIREANEDQSSSLMSLD^WS^WL^AE



B

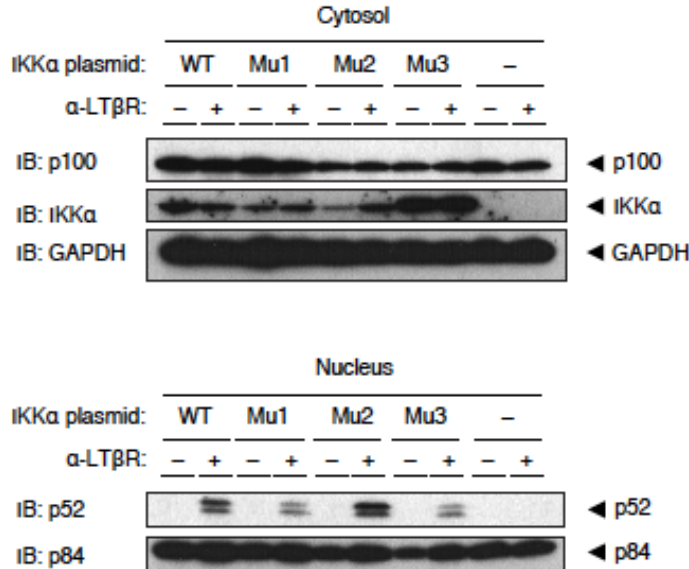


Fig. S3. LIR mutants of IKKα maintain intact kinase activity.

(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. Cyttoplasmic and nuclear extracts were prepared and analyzed by Western blotting with antibodies against the indicated proteins. Blots are representative of 3 independent experiments.

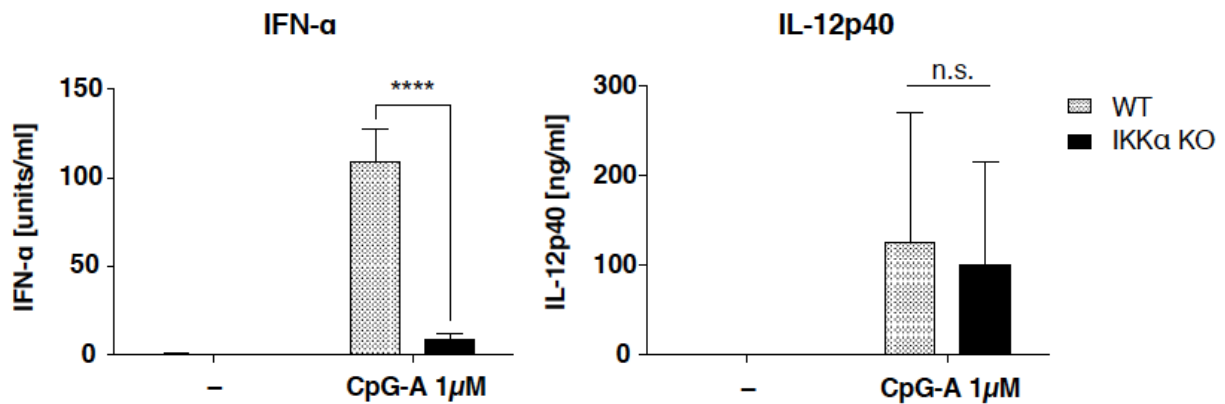


Fig. S4. *Fms*-related tyrosine kinase 3 ligand–derived *Ikkα*-deficient pDCs show impaired IFN- α production but intact IL-12p40 after CpG-A stimulation.

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 \pm SEM from 3 independent experiments. **, *** denote $P < 0.01$, $P < 0.001$, respectively.

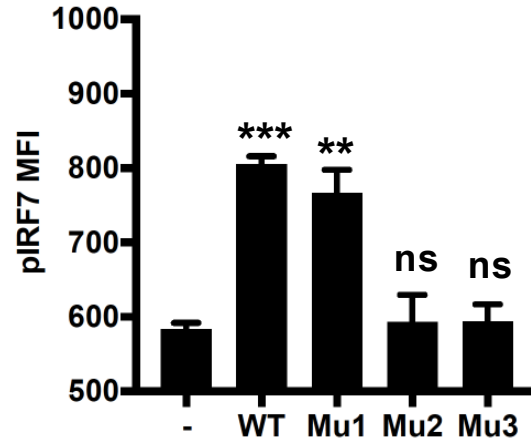


Fig. S5. WT and LIR-1 but not LIR-2 or LIR-3 mutant IKK α induces IRF7 phosphorylation.

HEK293T cells were transfected with IKK α -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 \pm SEM from 3 independent experiments.

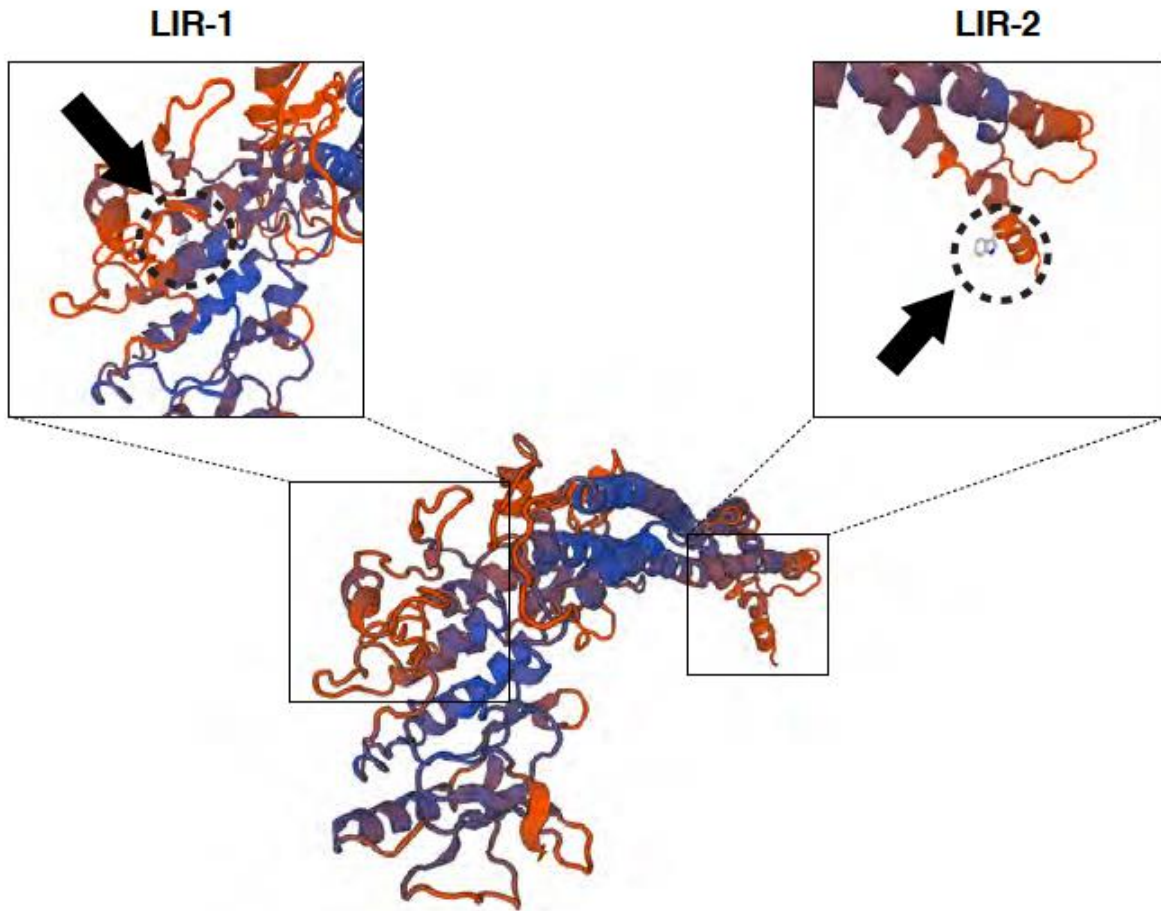


Fig. S6. LIR prediction based on IKK β structure by SWISS-MODEL.

Two LIRs (LIR-1, LIR-2) of IKK α 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.