Expanded View Figures

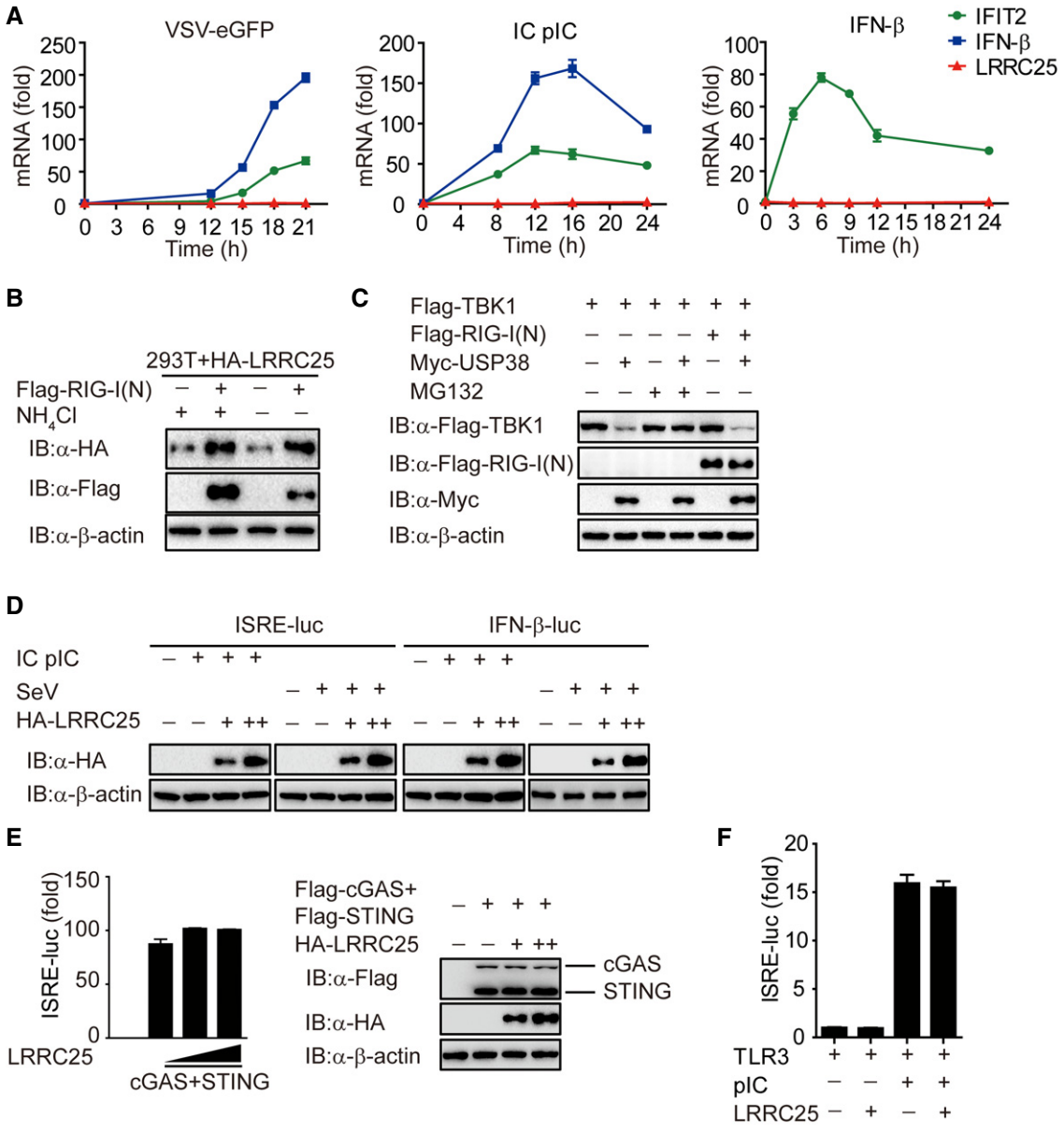

Figure EV1. LRRC25 has no effect on the type I IFN signaling pathway mediated by TLR3 or c-GAS, related to Fig 1.

A THP-1 cells were treated with VSV-eGFP (MOI = 0.1), intracellular (IC) poly(I:C) low molecular weight (5 μg/ml) or IFN-β (10 ng/ml) for indicated time points. Total RNA was extracted for qPCR analysis for *LRRC25*, *IFN-β*, and *IFIT2*.

B HEK293T cells were transfected with plasmids for *LRRC25*, together with an empty vector or *RIG-I (N)* for 24 h. Before harvesting, the cells were treated with DMEM or NH_4Cl (10 mM) for 6 h. Cell lysates were used for immunoblot analysis with the indicated antibodies.

C HEK293T cells were transfected with *Flag-TBK1*, together with an empty vector, *Flag-RIG-I (N)*, or *Myc-USP38* for 24 h. Before harvesting, the cells were treated with DMSO or MG132 (10 μM) for 6 h. Cell lysates were harvested and used to perform immunoblot analysis with the indicated antibodies.

D The expression of LRRC25 in Fig 1F and G was analyzed by IB analysis.

E HEK293T cells were transfected with an empty vector (no wedge) or increasing amounts (wedge) of vector for *LRRC25*, along with vectors for *cGAS* and *STING*. 24 h post-transfection, the cells were analyzed for ISRE activity by a reporter gene assay, and the expressions of cGAS, STING, and LRRC25 were analyzed by IB analysis.

F HEK293T cells were transfected with an empty vector or plasmid for *LRRC25*, plus plasmids for TLR3 and *ISRE-luc* reporter, followed by no treatment or treatment with poly (I:C) (10 μg/ml). After 24 h, cell lysates were analyzed for ISRE-luc activity.

Data information: In (B–E), data are representative of three independent experiments. In (A, E, F), data are mean values ± SEM (n = 3).

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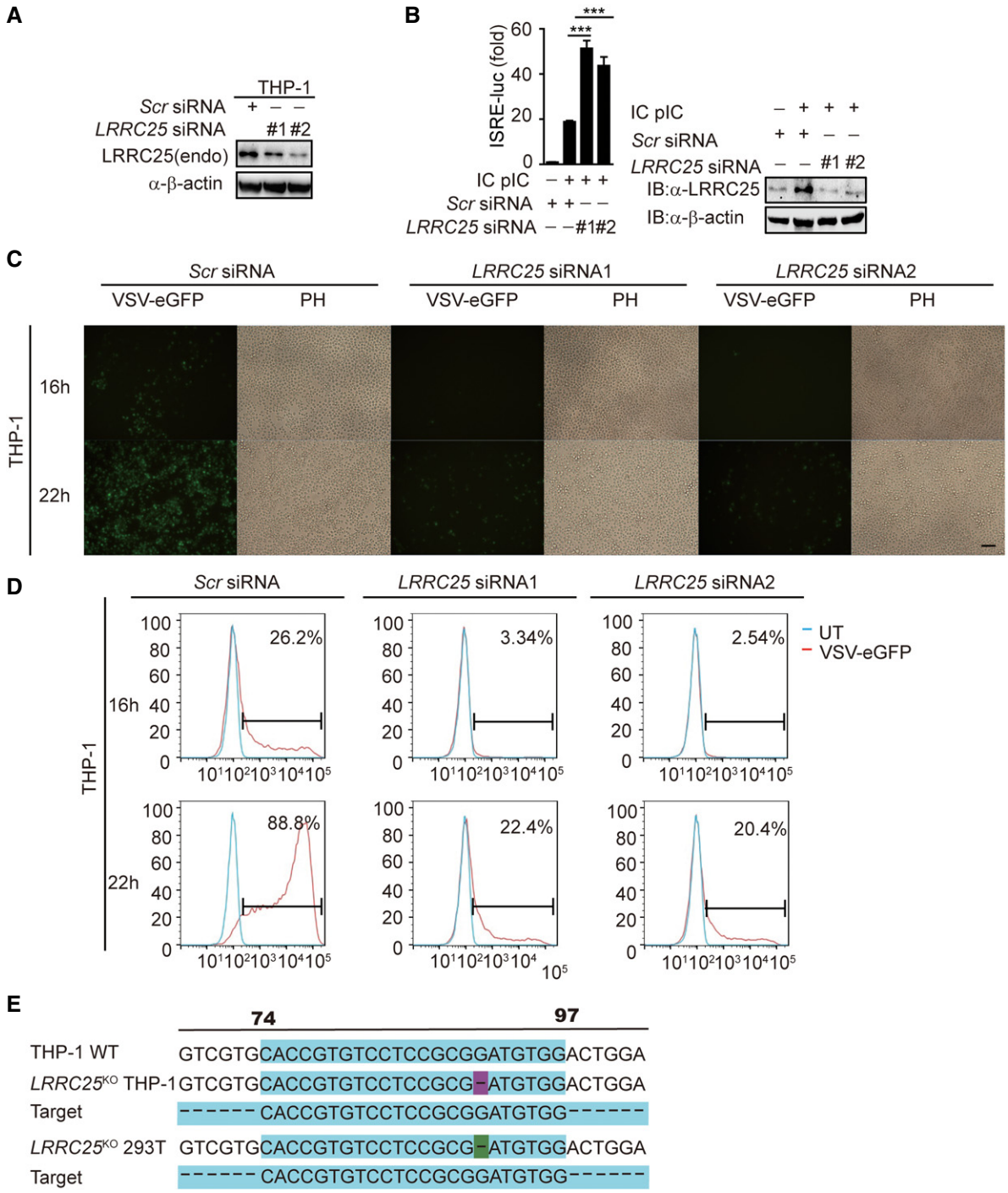

Figure EV2. LRRC25 deficiency enhances antiviral immune responses, related to Fig 2.

A THP-1 cells were transfected with control or *LRRC25*-specific siRNAs. Cell lysates were harvested and used to perform immunoblot analysis with the indicated antibodies.

B HEK293T cells were transfected with control or *LRRC25*-specific siRNAs, together with an *ISRE-luc* reporter plasmid. After 24 h, the cells were treated with IC poly(I:C) (5 μg/ml) for 24 h. The cells were analyzed for ISRE activity by a reporter assay, and the expression of LRRC25 was analyzed by IB analysis.

C THP-1 cells were transfected with control or *LRRC25*-specific siRNAs for 24 h, and then, the cells were infected with VSV-eGFP (MOI = 0.01) for the indicated time points and subjected to phase-contrast (PH) and fluorescence microscopy analyses. Scale bar, 80 μm.

D Flow cytometry analyses of THP-1 cells in (C). Numbers at the top-right corner indicate the percentage of cells expressing eGFP (infected cells).

E *LRRC25* KO THP-1 and *LRRC25* KO HEK293T cells were generated by the CRISPR/Cas9 system. The sequences of target sgRNA are as indicated.

Data information: In (A–D), data are representative of three independent experiments. In (B), data are mean values ± SEM (n = 3). ***P < 0.001 (Student's t-test).

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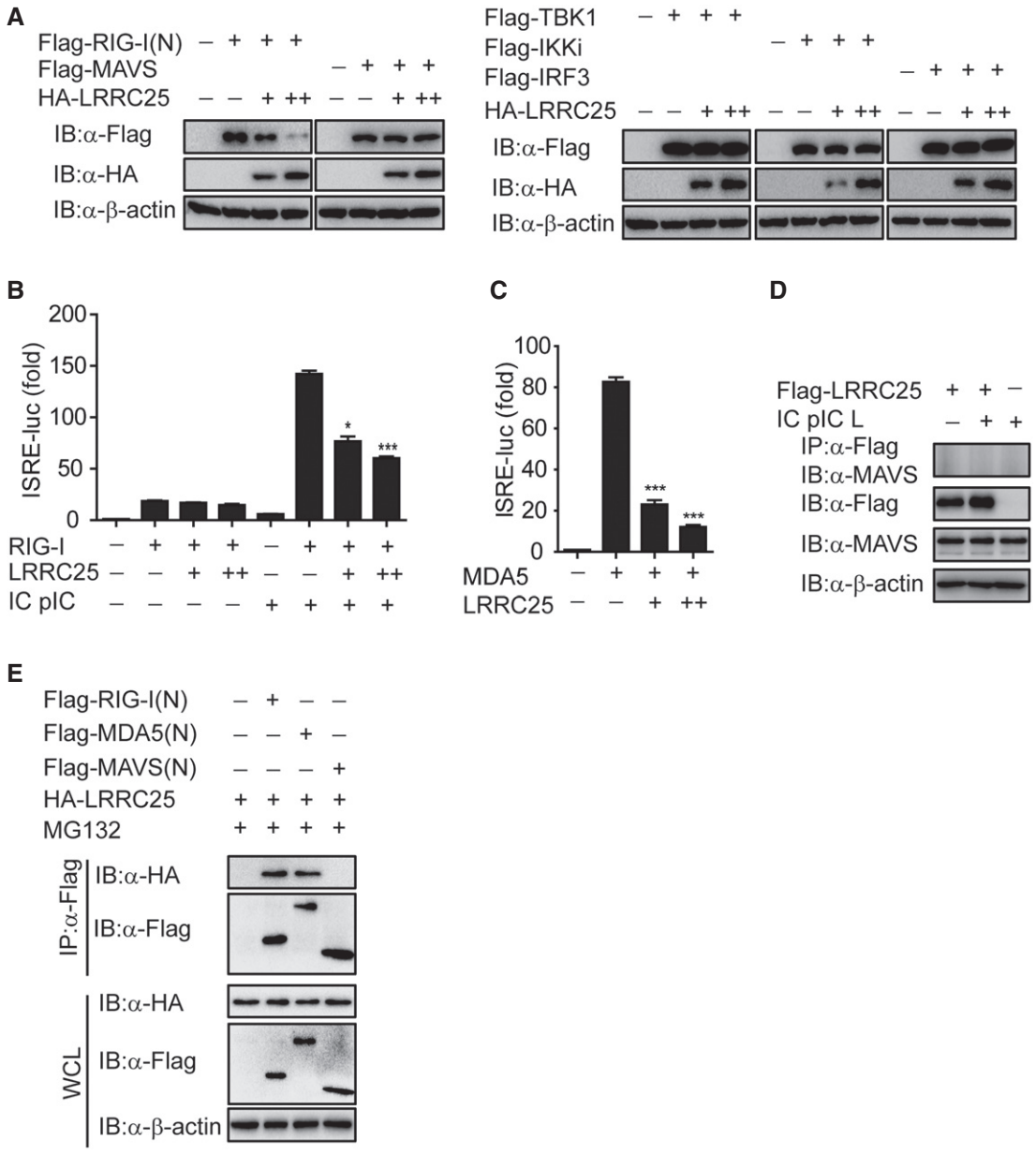

Figure EV3. LRRC25 cannot interact with MAVS, related to Fig 3.

A The expressions of RIG-I (N), MAVS, TBK1, IKKi, IRF3, and LRRC25 in Fig 3A were analyzed by IB analysis.

B HEK293T cells were transfected with an empty plasmid or increasing amounts of plasmid for *LRRC25*, plus an *ISRE-luc* reporter and plasmids for *RIG-I*. After 12 h, cells were left untreated or treated with IC poly(I:C) (5 μg/ml) for 24 h. Cell lysates were analyzed for ISRE-luc activity.

C HEK293T cells were transfected with an empty plasmid (no wedge) or increasing amounts (wedge) of plasmid for *LRRC25*, plus an *ISRE-luc* reporter and plasmid for *MDA5*. 24 h post-transfection, cell lysates were analyzed for ISRE-luc activity.

D HEK293T cells were transfected with *Flag-LRRC25*. After 12 h, the cells were left untreated or treated with IC poly(I:C) LMW (5 μg/ml) for 24 h. Cell lysates were immunoprecipitated using anti-Flag, followed by immunoblots using the indicated antibodies.

E HEK293T cells were transfected with *Flag-RIG-I (N)*, *Flag-MDA5 (N)*, *Flag-MAVS (N)*, and *HA-LRRC25* for 24 h. Before harvesting, the cells were treated with DMSO or MG132 (5 μM) for 4 h. Cell lysates were immunoprecipitated using anti-Flag, followed by immunoblot using the indicated antibodies.

Data information: In (A, D, E), data are representative of three independent experiments. In (B, C), data are mean values ± SEM ($n = 3$). *$P < 0.05$, ***$P < 0.001$ (Student's *t*-test).
Source data are available online for this figure.

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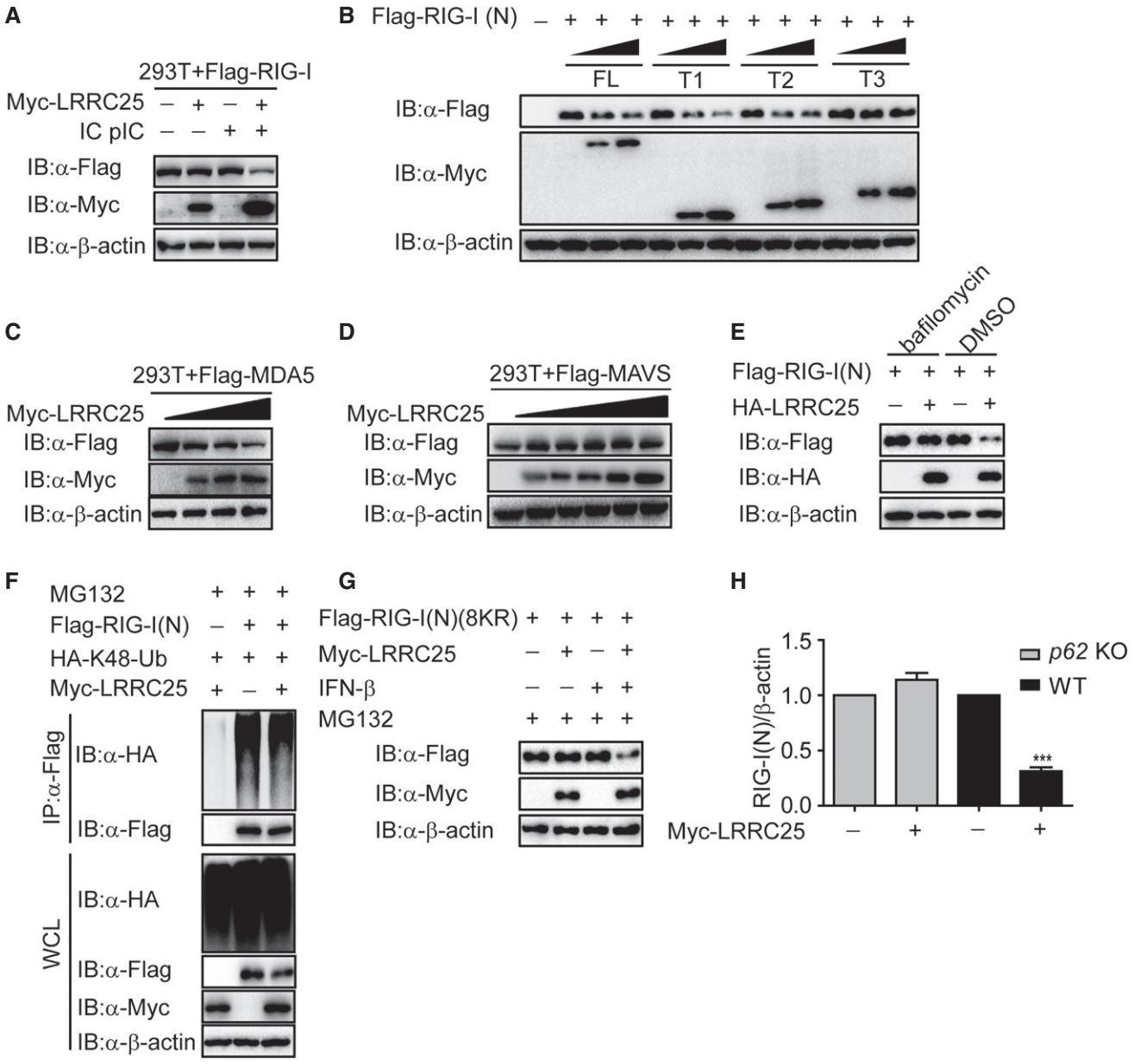

Figure EV4. LRRC25 promotes the degradation of RIG-I (N) and MDA5 but not MAVS, related to Figs 4 and 5.

A HEK293T cells were transfected with *Flag-RIG-I* together with an empty vector or *Myc-LRRC25*. After 12 h, the cells were left untreated or treated with IC poly(I:C) (5 μg/ml) for 24 h. Cell lysates were used for immunoblot analysis with the indicated antibodies.

B The expressions of RIG-I (N) and deletion mutants of LRRC25 in Fig 4D were analyzed by IB analysis.

C HEK293T cells were transfected with *Flag-MDA5*, together with an empty plasmid (no wedge) or increasing amounts (wedge) of plasmid for *LRRC25*. 24 h post-transfection, cells were harvested and used to perform immunoblot analysis with the indicated antibodies.

D HEK293T cells were transfected with *Flag-MAVS* together with an empty vector or *Myc-LRRC25*. 24 h post-transfection, cells were harvested and used to perform immunoblot analysis with the indicated antibodies.

E HEK293T cells were transfected with plasmids for *Flag-RIG-I (N)*, together with an empty plasmid or HA-LRRC25. 18 h post-transfection, the cells were treated with DMSO or bafilomycin A1 (0.2 μM) for 6 h. Cell lysates were used for immunoblot analysis with the indicated antibodies.

F HEK293T cells were transfected with plasmids for *HA-K48-Ub* and *Flag-RIG-I (N)*, together with an empty vector or *Myc-LRRC25*. 24 h post-transfection, the cells were treated with MG132 (5 μM) for 4 h. Cell lysates were immunoprecipitated using anti-Flag, followed by immunoblots using the indicated antibodies.

G HEK293T cells were transfected with plasmids for *Flag-RIG-I (N)(8KR)* together with an empty vector, or *Myc-LRRC25* for 24 h. Before harvesting, the cells were treated with IFN-β (10 ng/ml) and MG132 (5 μM) for 8 and 4 h, respectively. Cell lysates were harvested and used to perform immunoblot analysis with the indicated antibodies.

H Intensity analysis of the bands from the three independent experiments with the same setting in Fig 5E.

Data information: In (A–G), data are representative of three independent experiments. In (H), data are mean values ± SEM (n = 3). ***$P < 0.001$ (Student's t-test).

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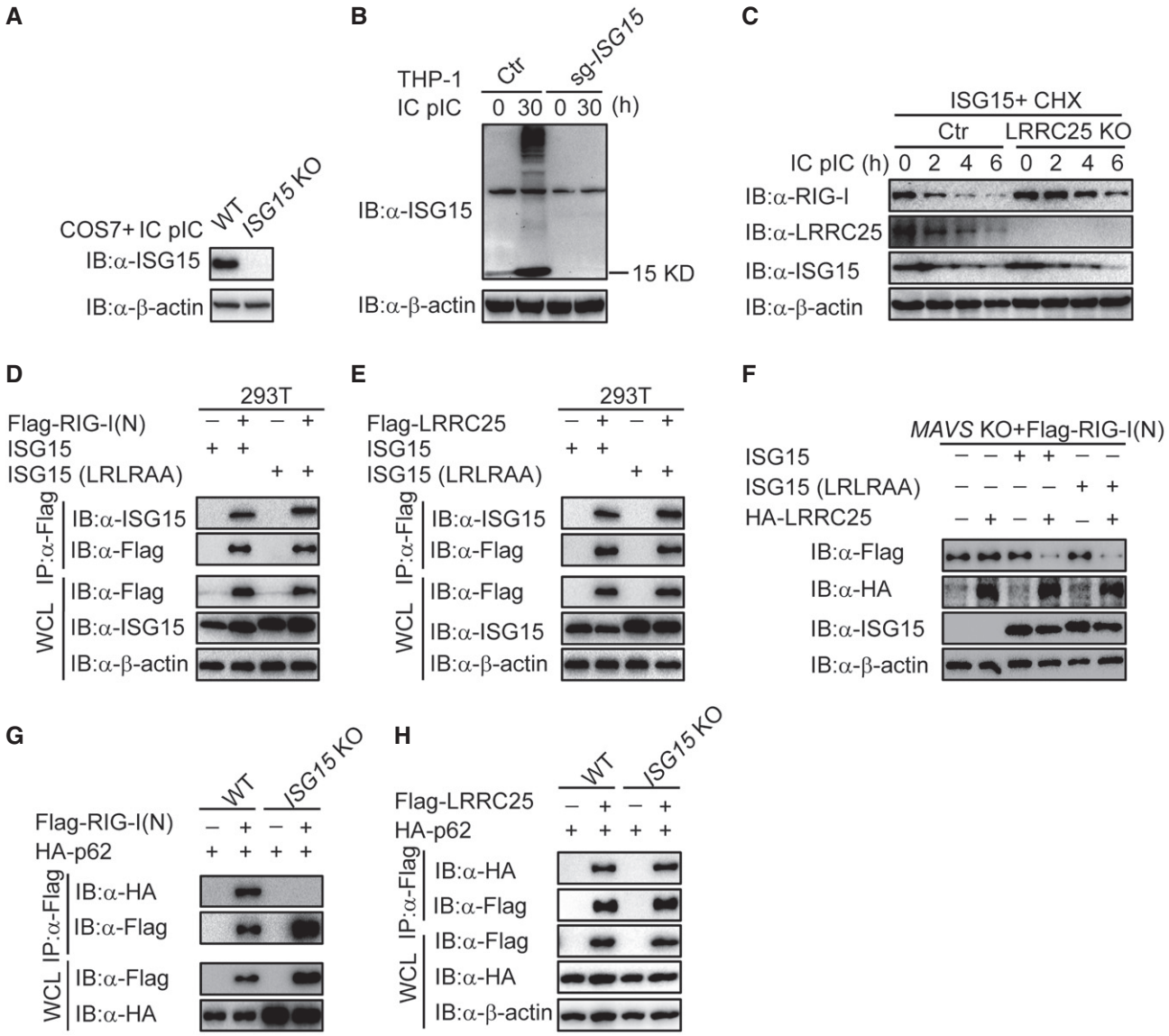

Figure EV5. RIG-I (N) and LRRC25 interact with ISG15 in an unconjugated manner, related to Figs 6 and 7.

A, B Control, *ISG15* KO COS7, and KO THP-1 cells were left untreated or treated with IC poly(I:C) (5 μg/ml) for 24 or 30 h, respectively. Cell lysates were harvested and used to perform immunoblot analysis with the indicated antibodies.

C Control or *LRRC25* KO HEK293T cells were transfected with *ISG15*. After 12 h, the cells were pre-treated with CHX (100 μg/ml) for 2 h, followed by IC poly(I:C) (5 μg/ml) for indicated time points. Cell lysates were used for immunoblot analysis with the indicated antibodies.

D, E HEK293T cells were transfected with *ISG15* or *ISG15 (LRLRAA)*, together with *Flag-RIG-I (N)* (D) or *Flag-LRRC25* (E). 24 h post-transfection, cell lysates were immunoprecipitated using anti-Flag, followed by immunoblot using the indicated antibodies.

F *MAVS* KO HEK293T cells were transfected with *Flag-RIG-I (N)*, together with an empty vector, *ISG15*, *ISG15 (LRLRAA)*, or *HA-LRRC25*. 24 h post-transfection, cell lysates were harvested and used to perform immunoblot analysis with the indicated antibodies.

G WT and *ISG15* KO COS7 cells were transfected with *Flag-RIG-I (N)* and *HA-p62* for 24 h. Cell lysates were immunoprecipitated using anti-Flag, followed by immunoblots using the indicated antibodies.

H WT and *ISG15* KO COS7 cells were transfected with *Flag-LRRC25* and *HA-p62* for 24 h. Cell lysates were immunoprecipitated using anti-Flag, followed by immunoblots with the indicated antibodies.

Data information: In (A, B), data are representative of two independent experiments. In (C–H), data are representative of three independent experiments.
Source data are available online for this figure.

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