**Figure EV1.**
**Figure EV1. Structural analyses of p97-Npl4 complex in antiviral signaling.**

A. Schematic illustration of wild-type Npl4 and its truncation mutants.

B. IFNβ transactivity and IFNB transcription in cells transfected with the indicated plasmid. Error bars represent SD of data obtained in three independent experiments. One-way analysis of variance (ANOVA) was used. n.s., no significant difference; *P < 0.05; **P < 0.01; ***P < 0.001 in comparison with control group.

C, D. Alignments of p97 sequences and of Npl4 sequences of Drosophila, human, mouse, and S. pombe. Residues predicted to be critical for complex formation by the previous modeling are indicated by black asterisks; residues on the interface of the current structure of the Ter94-dNpl4 complex are indicated by red asterisks.

E. Structural comparison of complexes of p97 with various adaptors, including Npl4, p47 (PDB ID: 1S3S), FAF (PDB ID: 3QQ8), and OTU1 (PDB ID: 4KDI).

F. Structural comparison of dNpl4 in the Ter94-dNpl4 complex with the NMR structure of Npl4 (PDB ID: 2PJH).

G. Structural comparison of Ter94-dNpl4 with the modeled structure of p97-Npl4 (PDB ID: 2PJH).

H. Octet Red 96 analysis between p97 and Npl4 mutants.

I. Octet Red 96 analysis between Npl4 and p97 mutants.

Data information: For abbreviations, see Figs 1 and 2.
Related to Fig 3.

![Image of Figure EV1](image_url)

**Figure EV2. p97-Npl4 complex inhibits RIG-I ubiquitination.**

A. RIG-I accumulation and IRF3 phosphorylation in poly(I:C)-treated cells after transfection with different shRNAs.

B. RIG-I accumulation and IRF3 phosphorylation in SeV-infected cells after transfection with different siRNAs.

C. Transcriptional levels of RIG-I in cells overexpressing Npl4 after stimulation with poly(I:C). Error bars represent SD of data obtained in three independent experiments.

D. Ubiquitination of RIG-I in sip97 cells transfected with ubiquitin and wild-type or 3A mutant p97.

E. Ubiquitination of RIG-I in cells transfected with ubiquitin and wild-type or 4A mutant Npl4.

Data information: For abbreviations, see Figs 1 and 3.
Related to Fig 4.
**Figure EV3.** RNF125 is essential for RIG-I degradation by Npl4.

A Q–PCR analysis of knockdown efficiency of RNF125 and c-Cbl in HEK293T cells. Error bars represent SD of data obtained in three independent experiments. Student's t-test was used. n.s., no significant difference; **P < 0.01; ***P < 0.001 in comparison with control group.

B Ubiquitination of RIG-I in RNF125-knockdown cells after transfection with Npl4.

C Ubiquitination analysis of RIG-I-CARDs and its K181R mutant in cells transfected with ubiquitin and Npl4.

D Residues T170, K172, and K181 in the structure of RIG-I CARDs oligomer in complex with K63-linked di-ubiquitin (Ub) (PDB ID: 4NQK). Residues T170, K172, and K181 are highlighted as spheres.

Related to Fig 5.
Figure EV4. Npl4 directly interacts with RIG-I.
A. In vitro pulldown assay to assess the direct interaction between the tandem CARDs of RIG-I and Npl4.
B. Co-IP assay for Npl4 and wild-type or mutant RIG-I-CARDs in cells transfected with Npl4, ubiquitin, and TRIM25.
C. Direct interaction between Npl4 and RIG-I with or without K63 ubiquitination.
Related to Fig 6.
Source data are available online for this figure.
Figure EV5. Inhibition of p97-Npl4 protects mice from viral infection.

A. Analysis of IFNβ production in mice treated with DBeQ or NMS-873.
B. Weight change of mice after treatment with DBeQ and NMS-873.
C. A working model for p97-Npl4 regulation of RIG-I-mediated antiviral signaling.

Data information: Error bars represent SD of data obtained in three independent experiments. Student’s t-test was used. n.s., no significant difference; *P < 0.05; **P < 0.01 in comparison with control group.

Related to Fig 7.