Regulation of Toll-like receptor signaling by NDP52-mediated selective autophagy is normally inactivated by A20

Megumi Inomata,[1] Shumpei Niida,[2] Ken-ichiro Shibata,[3] and Takeshi Into[1]*

[1] Department of Oral Microbiology, Division of Oral Infections and Health Sciences, Asahi University School of Dentistry, Hozumi 1851, Mizuho, Gifu 501-0296, Japan; [2] Laboratory of Genomics and Proteomics, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, Obu, Aichi 474-8522, Japan; [3] Laboratory of Oral Molecular Microbiology, Department of Oral Pathobiological Science, Hokkaido University Graduate School of Dental Medicine, Sapporo 060-8586, Japan.

*Corresponding author. Mailing address: Department of Oral Microbiology, Division of Oral Infections and Health Sciences, Asahi University School of Dentistry, Hozumi 1851, Mizuho, Gifu 501-0296, Japan. Phone & Fax: 81 58 3291422. E-mail: into@dent.asahi-u.ac.jp

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Supplementary materials and methods

Fig. S1 Etoposide promotes formation of autophagic vacuoles in human bone marrow mononuclear cells.

Human bone marrow-derived primary mononuclear cells were stimulated with 25 μg/ml Etoposide for 20 h. Then the cells were assessed by electron microscopy. Arrows: autophagic vacuoles. Arrowheads: autophagosomes. The magnified photo is the area indicated by the squares. Scale bar: 2 μm. Original magnification, ×3000. For each cell, the autophagic area was calculated by expressing the total area of autophagic vacuoles as a percentage of the cytoplasmic area. Error bars indicate ± SD (n=5). *: $P < 0.01$.

Fig. S2 HeLa cells are adequately responsive to Bafilomycin A1.

HeLa cells were treated with 100 nM Bafilomycin A1 for 6 h. Cells lysates were then analyzed by IB with indicated antibodies. Results are representative of three independent experiments.

Fig. S3 NDP52 degrades overexpressed TRIF.

HEK293T cells were transfected with Flag-TRIF together with indicated amounts of HA-NDP52 for 24 h. Cells lysates were obtained by using lysis buffer containg 0.5% Triton X-100 followed by clarification or by using 4% LDS sample buffer without clarification. Samples were analyzed by IB with

indicated antibodies. Results are representative of three independent experiments.

Fig. S4 Sequence alignment of vertebrate NDP52.

Amino acid sequences of NDP52 of humans, chimpanzees, bovines, dogs, mice, chickens and zebrafishes are shown. Corresponding residues that match human NDP52 are shown with the grey background. Also, domains of human NDP52, SKICH domain (1-127, red), coiled-coil domain with leucine zipper sequence (134-350, green) and LIM-like domain (395-446, blue), are shown.

Fig. S5 NDP52 interacts with and degrade MyD88.

a HEK293T cells were transfected with Flag-MyD88 and pNF-κB-Luc together with indicated amounts of HA-NDP52 for 24 h. Luciferase activity was measured. Data are expressed as the mean ±SD (n=3).

b HEK293T cells were transfected with Flag-MyD88 together with indicated amounts of HA-NDP52 for 24 h. Cells lysates were obtained by using lysis buffer containg 0.5% Triton X-100 followed by clarification or by using 4% LDS sample buffer without clarification. Samples were analyzed by IB with antibodies

to Flag epitope and HA epitope.

c HEK293T cells were transfected with Flag-MyD88 together with either HA-NDP52 or empty vector for 18 h. Cells were treated with either 20 μM MG-132 or 10 mM 3-MA for 6 h. Cell lysates were

analyzed by IB with antibody to Flag epitope.

d HEK293T cells were transfected with Flag-MyD88 together with HA-NDP52 or empty vector for 24 h. IP with anti-Flag-agarose was carried out with clarified cell lysates, followed by IB with antibodies to HA epitope and Flag epitope.

e HEK293T cells were cotransfected with Flag-MyD88 and HA-NDP52, HA-tagged NDP52 mutant or empty vector for 24 h. IP with anti-Flag agarose was carried out with clarified cell lysates, followed by IB with antibodies to HA epitope and Flag epitope. All results are representative of three independent experiments.

Fig. S6 Sequence alignment of the C-terminal regions of human NDP52, COCOA and TAX1BP1. Cysteine residues are shown in red letters. The Pro-Pro-X-Tyr motifs of TAX1BP1 are shown in the boxes. The C-terminal LIM-like domain of NDP52 is analogous to the N-terminal regions of CRP, CRIP1 and CRIP2.