**Supplemental Methods**

**Cell stimulation and reagents**

Jurkat lymphocytes, ABC DLBCL cells (OCI-Ly3, OCI-Ly10, U2932, and RIVA), GCB DLBCL cells (OCI-Ly7, OCI-Ly19, and BJAB) and Peripheral Blood Mononuclear Cells from healthy donors (Etablissement Francais du Sang) were used. Cell stimulation was achieved with 20 ng.ml\(^{-1}\) Phorbol 12-myristate 13-acetate (PMA, Sigma) plus 300 ng.ml\(^{-1}\) ionomycin (Calbiochem), or with 1 µg.ml\(^{-1}\) of both anti-CD3 and anti-CD28 antibodies (BD Biosciences), or with 10 ng.ml\(^{-1}\) of tumor necrosis factor-alpha (TNF\(\alpha\), R&D systems). Mass spectrometry analysis was obtained from Nextgen Sciences (Ann Arbor, MI). DiOC\(_6\), Annexin V, and propidium iodide were from Invitrogen.

**Cells transfection and cells transduction**

Human lymphoma cell lines and Jurkat cells were transfected by electroporation (BTX ECM 830, Harvard Apparatus), and primary lymphocytes were nucleofected (Amaxa D4, Lonza). Luciferase gene reporter assays, ELISA, and RT-PCR were performed as previously described.\(^1\)\(^2\) siRNA sequences (Invitrogen) were:

CARMA1, UGUCCCGUUGGACACAUGCACAAAA;
HOIP.1, GGUACUGGCGUGUGUGCAAGUUUA;
HOIP.2, GAGAUGUGGUGCGAUUAUGGCUA;
HOIP.3, CACCACCCUCGAGACUGGCCUCUUCU;
HOIL-1, GGCAGCGACGCAGCGCGUGGAAGAUCC;
SHARPIN, UGCCUGACGCGGUGUGCCUCUCUAA;
OTULIN.1, GCGGAGGAAUAUAUGCCUCUAUGAAG;
OTULIN2, UCUCCAAGUACAACACGGAAGAAUU;
USP34, GGCAAGACAUUUGGCUGACUGUAUU.

shRNA sequences in GFP-expressing GIPZ lentiviral vectors (Thermo Scientific) were TCTGCTCTATCCTACTCGGCA (V3LHS-399212), or nonsilencing control. For cells transduction, lentivirus particles were produced in HEK 293T cells by cotransfecting pGIPZ constructs together with pVSV-G and psPAX2 plasmids. Supernatants containing lentiviral particles were collected after 48 and 72 hours, concentrated by a 120,000g ultracentrifugation for 90 min and incubated with lymphoma cell lines in the presence of 8 µg.ml⁻¹ polybrene (Santa Cruz). Fraction of GFP-positive cells over time following retroviral infection was determined by flow cytometry (BD). The lentiviral shRNA vector, pTY-shRNA-EF1a-puroR-2a-GFP-Flag, was utilized to silence and rescue human HOIP in human cells as described previously. The shRNA sequence against human HOIP (sense strand sequence, 5’-GGCGTGCTGGTCAGTCTAA-3’) was cloned into the vector after the U6 promoter. RNAi-resistant cDNA sequences encoding wild-type human HOIP and catalytically disabled HOIP (C699S/C702S) were cloned into the vector to replace GFP for rescuing HOIP expression on top of HOIP knockdown. Lentiviral infection was described previously.

**Immunoblotting, Immunoprecipitation, and subcellular fractionation**

Cells were lysed with TNT buffer (50 mM TRIS pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% NP-40, 2 mM EDTA, protease inhibitors (Roche)) for 30 min on ice. Samples were cleared by centrifugation at 9,000g and proteins concentration was determined by BCA (Pierce). 10-20 µg proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes (Hybond, Amersham). For immunoprecipitation experiments, samples were first precleared with Protein G
agarose (Roche) for 30 min and then incubated with 5 µg antibodies and Protein G agarose for 1 hour at 4°C. To analyze NEMO ubiquitination status, cells were lysed with 50 mM TRIS pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM NaVO₄, 1 mM NaF, protease inhibitors, containing 1% SDS and 1 µl benzonase (250U/µl, Novagen). Samples were boiled for 10 min at 90°C and SDS was diluted to reach a final concentration of 0.1% prior immunoprecipitation. For cytosolic and nuclear isolation, cells were lysed with 200 µL of buffer A (10 mM Hepes pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM Na₃VO₄, 1 mM NaF, and protease inhibitors) with 12.5 µL NP-40 10% on ice for 5 min. Nuclei were spun at 2,500g for 3 min, lysed with 30 µl buffer C (20 mM Hepes pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM Na₃VO₄, 1 mM NaF, and protease inhibitors), and cleared by a 12,000g centrifugation. Antibodies used were: anti-BCL10, anti-CK1α, anti-MALT1, anti-NEMO, anti-p65, anti-TNFR, anti-tubulin, anti-ubiquitin (Santa Cruz), anti-CARMA1, anti-P-ERK, anti-P-IκBα, anti-P-IKK (Cell Signaling Technology), anti-HOIL-1, anti-HOIP, anti-GAPDH (Sigma), anti-HOIP, anti-SHARPIN (Bethyl Laboratories), anti-IKKβ, and anti-NEMO (BD), anti-M1-linked ubiquitin (Genentech, Ref.5).

Confocal microscopy

Cells were placed onto poly-L-lysine-coated slides (Fisher Scientific) to adhere, fixed with 4% paraformaldehyde in PBS 1X (Electron Microscopy Sciences) for 10 min, and permeabilized for 5 min with 0.05% triton in PBS 1X (Sigma-Aldrich). Non-specific sites were blocked with 10% FCS in PBS 1X for 30 min. Antibodies against NF-κB p65 (C-20, Santa Cruz) were diluted in PBS 1X containing 0.5% BSA for 45 min. After washes with PBS 1X, cells were incubated with antibody conjugated to Alexa Fluor 488 (Life Technologies) for 30 min. Nuclei were illuminated
with 4',6'-diamidino-2-phenylindole (DAPI, Sigma). Coverslips were sealed with Fluoromount (Southern Biotech) and samples were analyzed at room temperature using a Leica confocal microscope SP5 with a 63x1.6 oil-immersion objective (Leica Microsystems, Wetzlar, Germany). Pictures were processed using Photoshop (Adobe Systems).

**Statistical analysis**

Statistical significance was assessed with two-way ANOVA tests with post hoc Tukey’s analysis (Prism GraphPad Software), and P values are indicated in the figure legends.

**Supplementary References**

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**Table S1.** Known partners of the CK1α-CBM complex identified in a mass spectrometry screen using CK1α as a bait.

**References**

Supplemental Figures

**Figure S1.** Cell lysates from Jurkat cells stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin for 15 min were immunoprecipitated with antibodies against CK1α. Samples were resolved on a 4-12% Bis-Tris gel. 24 gel slices were removed and further processed on a ProGest. Once digested with trypsin, segments were analyzed using LC/MS/MS with a 1h gradient on a LTQ Orbitrap XL mass spectrometer. Product ion data were searched against the concatenated forward and reverse IPI Human v3.79 protein database. The Mascot search engine was employed. (A) Amino acid sequence of HOIP. Shown in bold and underlined are the two peptides identified as CK1α-associated proteins by a mass spectrometry screen. (B) Mass spectrometry spectra of HOIP detected peptides ( Scaffold software).

**Figure S2.** Jurkat T lymphocytes were stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I) for 0 and 15 min. Cell extracts were prepared and immunoprecipitated (IP) with antibodies against HOIL-1, SHARPIN, or with control rabbit Ig (control) and analyzed by immunoblot (IB). Lys, lysates. Molecular weight markers (kDa) are shown. IB are representative of three independent experiments.
Figure S3. Full pictures of some immunoblots presented on Figure 1. Close circle shows the proteins of interest, (*) indicates nonspecific bands, and (**) Ig chains. Residual bands from previous staining are indicated. Molecular weight markers (kDa) are shown. Ub, ubiquitin.
**Figure S4.** (A) Jurkat T lymphocytes were stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I) for 0, 10 and 20 min. Cell extracts were prepared and immunoprecipitated (IP) with antibodies against CK1α and analyzed by immunoblot (IB) as indicated. Lys., lysates; Ub, ubiquitylated MALT1; (*) indicates nonspecific bands; (**) Ig heavy chains, and close circle the protein of interest. Molecular Weight markers (kDa) are indicated. IB are representative of three independent experiments.

**Figure S5.** (A, B) BJAB B cells were stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I) for 0 and 20 min. Lysates were immunoprecipitated (IP) with antibodies to CK1α (A) or SHARPIN (B), and immunoblots were performed as indicated. Symbol indicates the proteins of interest. Molecular weight markers (kDa) are shown. Data is representative of two independent experiments.

**Figure S6.** Confocal microscopy micrographs of NF-κB in nonspecific control- (NS-) or HOIP-silenced human peripheral blood mononuclear cells (PBMC) stimulated with 1 μg.ml⁻¹ anti-CD3 and anti-CD28 (CD3/28). Nuclei were also stained with DAPI (in grey). Number indicates percentage of cells with p65 redistributed in the nucleus. Unst., unstimulated. Representative images from two independent experiments are shown.
Figure S7. Jurkat T cells were transfected with control nonspecific (NS) siRNA or with siRNA against HOIP. After four days, cells were stimulated with 1 μg.ml⁻¹ anti-CD3 and anti-CD28 (CD3/28) for 0, 60 and 90 min. mRNA levels of IL-2 and GAPDH were measured by RT-PCR. Shown is representative of two independent experiments.

Figure S8. Lysates from control nonspecific (NS-) and HOIP-silenced Jurkat cells stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I) were immunoprecipitated (IP) with antibodies to CK1α. Immunoblots were performed as indicated. Close circle shows the proteins of interest; Ub, ubiquitylated MALT1. Molecular weight markers (kDa) are indicated. Immunoblots are representative of two independent experiments.

Figure S9. Jurkat T lymphocytes were stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I) for 0 and 20 min. Cell extracts in lysis buffer containing 1% SDS were boiled for 10 min. Samples were diluted ten times with lysis buffer without SDS prior immunoprecipitation (IP) with antibodies against NEMO. Immunoblots were performed as indicated. Lys., lysates. Molecular Weight markers (kDa) are indicated. Note that IKKβ, MALT1 and HOIP binding to NEMO was not detectable when SDS and heat were use. Immunoblots are representative of at least two independent experiments.
Figure S10. NF-κB luciferase reporter assays of Jurkat cells transfected with siRNA as indicated and stimulated with 1 μg.ml⁻¹ anti-CD3 and anti-CD28 (3/28) or with 10 ng.ml⁻¹ TNFα (mean ± SEM from three independent experiments; ns, non significant; **** P<0.0001 by ANOVA). Unst, unstimulated; RLU, relative light units. Immunoblots of cell lysates from NS-, HOIP-, HOIL-1- and SHARPIN- silenced cells were performed as indicated. Molecular weight markers (kDa) are shown. Data are representative of more than three independent experiments.

Figure S11. (A, B) Jurkat T lymphocytes were stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I) or with 10 ng.ml⁻¹ TNFα for 0, 10 and 20 min. Cell extracts were prepared and immunoprecipitated (IP) with antibodies to SHARPIN (A), CK1α or TNFR (B). Immunoblots were performed as indicated. Lys., lysates; Molecular Weight markers (kDa) are indicated. Immunoblots are representative of two independent experiments.
Figure S12. NF-κB gene reporter assay in Jurkat cells transfected with a nonspecific (NS) siRNA or with siRNA against OTULIN (two individual sequences), USP34 and HOIP. Cells were stimulated with 1 μg.ml⁻¹ anti-CD3 and anti-CD28 (CD3/28) or with 10 ng.ml⁻¹ TNFα. Data are mean ± SEM from three independent experiments (ns, non significant; ***P < 0.001; ****P < 0.0001 by ANOVA). Unst, unstimulated; RLU, relative light units. Silencing of the deubiquitylase USP34, which enhanced TCR- and TNFα-driven NF-κB activity, was used as a control.

Figure S13. (A) Jurkat cells were retrovirally infected to express GFP, GFP plus an shRNA against human HOIP (HOIPₕ), RNAi-insensitive HOIP-WT plus HOIPₕ, and catalytically inactive HOIP (HOIP-CS) plus HOIPₕ. Cells were stimulated with 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I), or with 10 ng.ml⁻¹ TNFα for 30 min. Shown are confocal microscopy micrographs of NF-κB p65 (red). A merged picture with nuclei staining is shown. (B) NF-κB luciferase reporter assay of cells as in (A) and stimulated with 0.5 μg.ml⁻¹ anti-CD3 and anti-CD28 (3/28), 20 ng.ml⁻¹ PMA plus 300 ng.ml⁻¹ ionomycin (P/I), or with 10 ng.ml⁻¹ TNFα. Histograms represent the mean ± SEM of three independent experiments (ns, non significant; ***P < 0.0001; ****P < 0.0001 by ANOVA).
Figure S14. (A) Cell lysates from ABC DLBCL lines (RIVA and U2932) and from GCB DLBCL lines (BJAB) were immunoprecipitated (IP) with antibodies to SHARPIN or CK1α and immunoblots (IB) were performed as indicated. Close symbol show HOIP and SHARPIN, respectively. (B) IP/IB as indicated of cell extracts from OCI-Ly10 (ABC DLBCL) and OCI-Ly19 (GCB DLBCL) lines. (*) indicates nonspecific bands, (**) Ig heavy chains, and close circle the protein of interest. IB are representative of two independent experiments.

Figure S15. (A) BJAB B cells stably expressing a control nonspecific shRNA (NS<sup>sh</sup>) or a shRNA against HOIP (HOIP<sup>sh</sup>) were stimulated with 20 ng.ml<sup>-1</sup> PMA plus 300 ng.ml<sup>-1</sup> ionomycin (P/l) and IB were performed as indicated. (B) U2932 cells were infected with a retrovirus that expressed a control nonspecific (NS<sup>sh</sup>) or a HOIP (HOIP<sup>sh</sup>) shRNA together with GFP. Shown is the fraction of GFP-positive cells over time relative to day 2 post-infection. Data is representative of three independent experiments.
Figure S16. (A) ABC DLBCL (OCI-Ly3) and GCB DLBCL (OCI-Ly19) were transfected with control nonspecific (NS) siRNA or with siRNA against the LUBAC (HOIP plus HOIL-1 plus SHARPIN) for 72h, and stained with DiOC₆ and propidium iodide (PI), or with Annexin V and PI. (B) ABC DLBCL (OCI-Ly3) and GCB DLBCL (BJAB) were transfected as in (A) with individual siRNA, and stained with DiOC₆ and PI. Data is representative of three independent experiments. (C) ABC DLBCL (OCI-Ly3 and OCI-Ly10) and GCB DLBCL (BJAB) were transfected as in (A) with NS or with siRNA against CARMA1, and stained with DiOC₆. (D) Extracts from cells as in (C) were analyzed by immunoblot as indicated. Molecular weight markers (kDa) are shown.
Figure S17. (A) Lysates from RIVA and U2932 cells transfected with control nonspecific (NS) siRNA or with siRNA against the LUBAC (HOIP plus HOIL-1 plus SHARPIN) for 72h were analyzed by IB as indicated. (B) Confocal microscopy micrographs of NF-κB p65 in NS- and LUBAC-silenced RIVA and U2932 as in (A). Nuclei were counterstained with DAPI. Representative images from three independent experiments are shown.