Supplementary Figures:

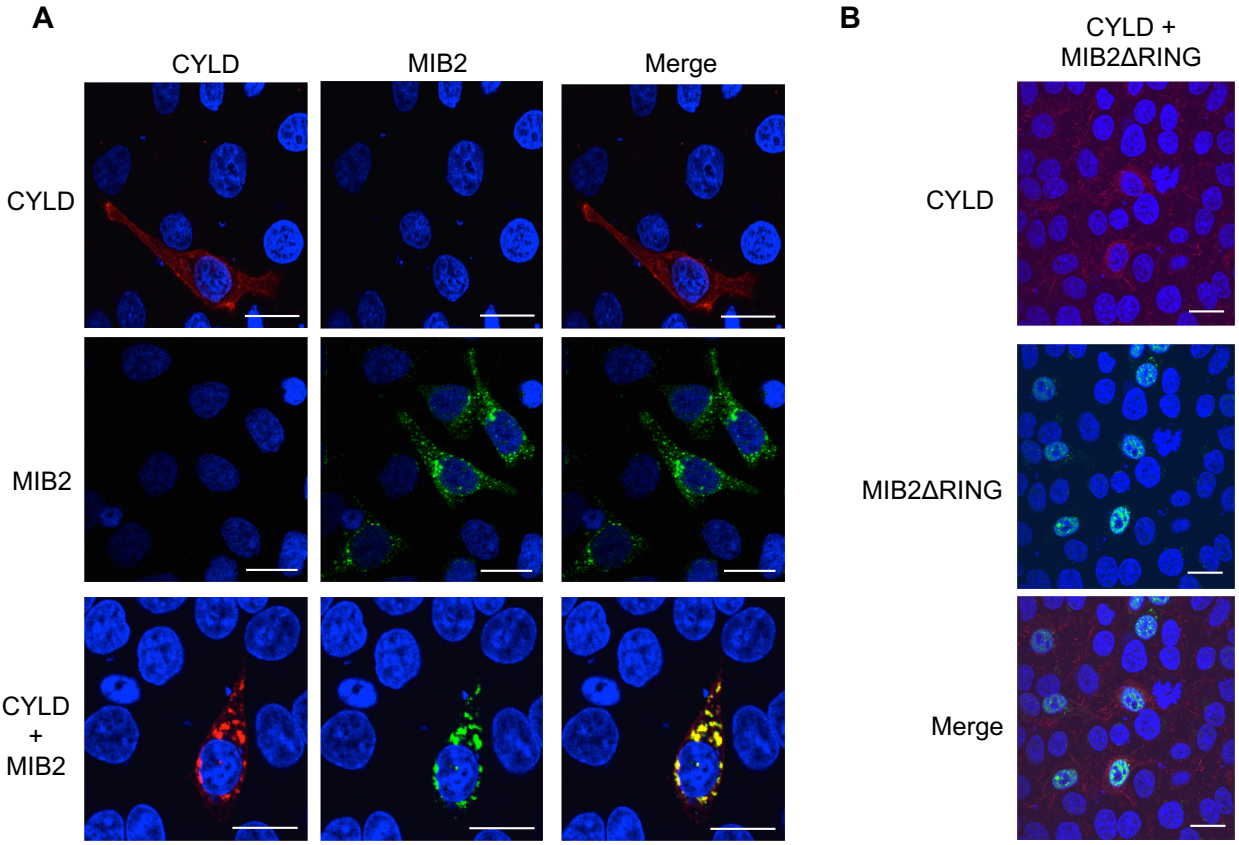

Figure S1. **MIB2 is co-localized with CYLD in the cytoplasm.** (A and B) Immunofluorescent staining of CYLD and MIB2. HeLa cells were co-transfected with the indicated FLAG-tagged CYLD along with V5-tagged wild-type MIB2 (A) or ΔRING-MIB2 (B), and immunofluorescent staining was performed using anti-FLAG or anti-V5 antibodies to visualize their intracellular localization. All scale bars denote 20 μM.

A

Description	#PSMs	Score	
CYLD {CYLD_Human}	6	169.04	
Sequence	**#PSMs**	**Exp Value**	**Modifications**
GSSSHNKPkATGSTSDPGNR	2	0.00229	K9(GG)
kALFVK	2	0.01021	K1(GG)

B

MSSGLWSQEKVTSPYWEERIFYLLLQECSVTDKQTQKLLKVPKGSIGQYIQDRSVGHSR
IPSAKGKKNQIGLKILEQPHAVLFVDEKDVVEINEKFTELLLAITNCEERFSLFKNRNRLSK
GLQIDVGCPVKVQLRSGEEKFPGVVRFRGPLLAERTVSGIFFGVELLEEGRGQGFTDGV
YQGKQLFQCDEDCGVFVALDKLELIEDDDTALESDYAGPGDTMQVELPPLEINSRVSLK
VGETIESGTVIFCDVLPGKESLGYFVGVDMDNPIGNWDGRFDGVQLCSFACVESTILLHI
NDIIPESVTQERRPPKLAFMSRGVGDKGSSSHNKPKATGSTSDPGNRNRSELFYTLNGS
SVDSQPQSKSKNTWYIDEVAEDPAKSLTEISTDFDRSSPPLQPPPVNSLTTENRFHSLPF
SLTKMPNTNGSIGHSPLSLSAQSVMEELNTAPVQESPPLAMPPGNSHGLEVGSLAEVK
ENPPFYGVIRWIGQPPGLNEVLAGLELEDECAGCTDGTFRGTRYFTCALKKALFVKLKSC
RPDSRFASLQPVSNQIERCNSLAFGGYLSEVVEENTPPKMEKEGLEIMIGKKKGIQGHY
NSCYLDSTLFCLFAFSSVLDTVLLRPKEKNDVEYYSETQELLRTEIVNPLRIYGYVCATKIM
KLRKILEKVEAASGFTSEEKDPEEFLNILFHHILRVEPLLKIRSAGQKVQDCYFYQIFMEKN
EKVGVPTIQQLLEWSFINSNLKFAEAPSCLIIQMPRFGKDFKLFKKIFPSLELNITDLLEDT
PRQCRICGGLAMYECRECYDDPDISAGKIKQFCKTCNTQVHLHPKRLNHKYNPVSLPK
DLPDWDWRHGCIPCQNMELFAVLCIETSHYVAFVKYGKDDSAWLFFDSMADRDGG
QNGFNIPQVTPCPEVGEYLKMSLEDLHSLDSRRIQGCARRLLCDAYMCMYQSPTMSLY
K

Figure S2. **Identification of MIB2-mediated ubiquitination sites in CYLD.** (A) Amino acid sequences identified by MS analysis with di-Gly ubiquitination signature. (B) Amino acid sequence of full-length CYLD. The underlined sequences indicate the amino acid sequences identified by MS analysis.

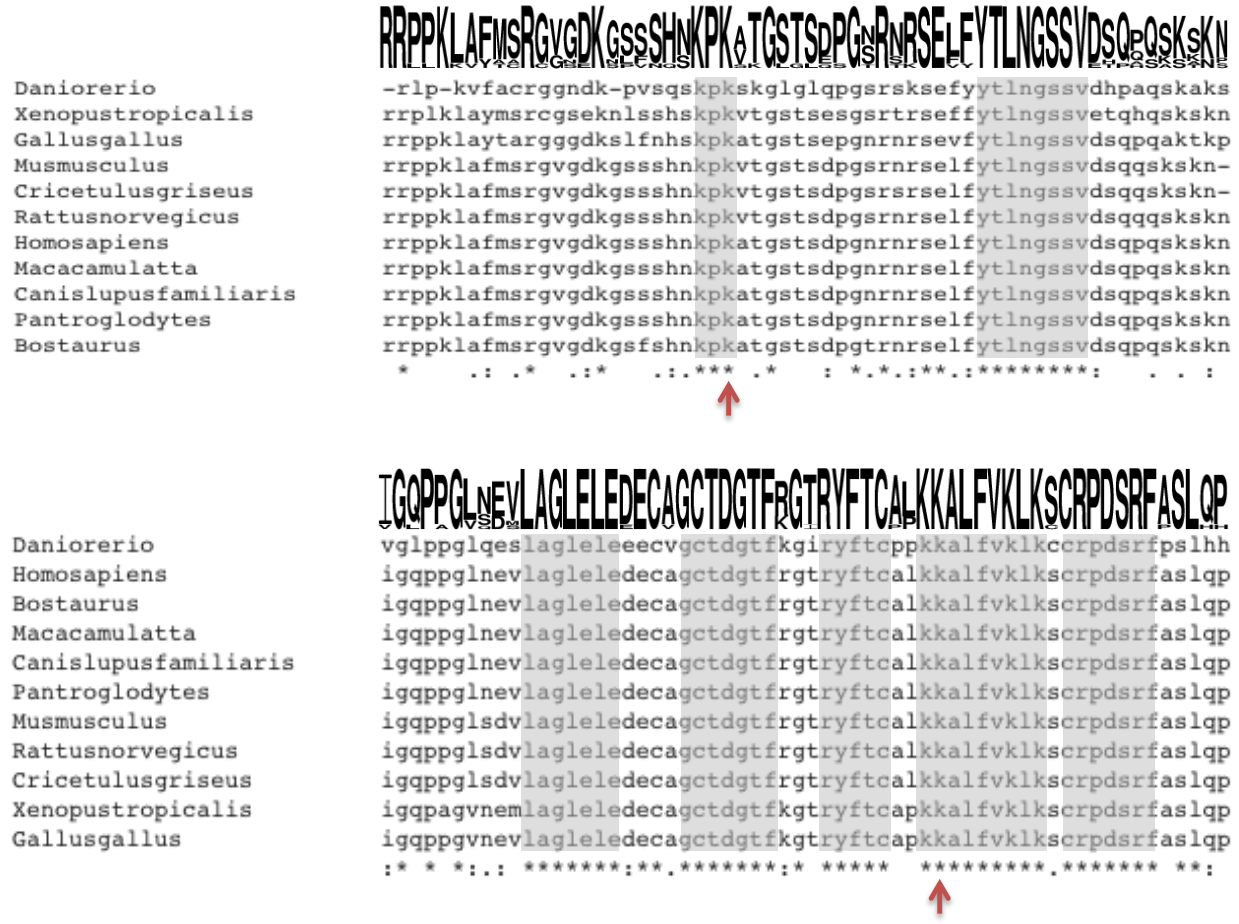

Figure S3. **Multiple-sequence alignment of conserved ubiquitination sites in CYLD orthologs.** The top logos show the conservation of residues. The arrows indicate ubiquitination site of CYLD. Upper and lower panels are sequences around human CYLD-K338 and -K530, respectively.

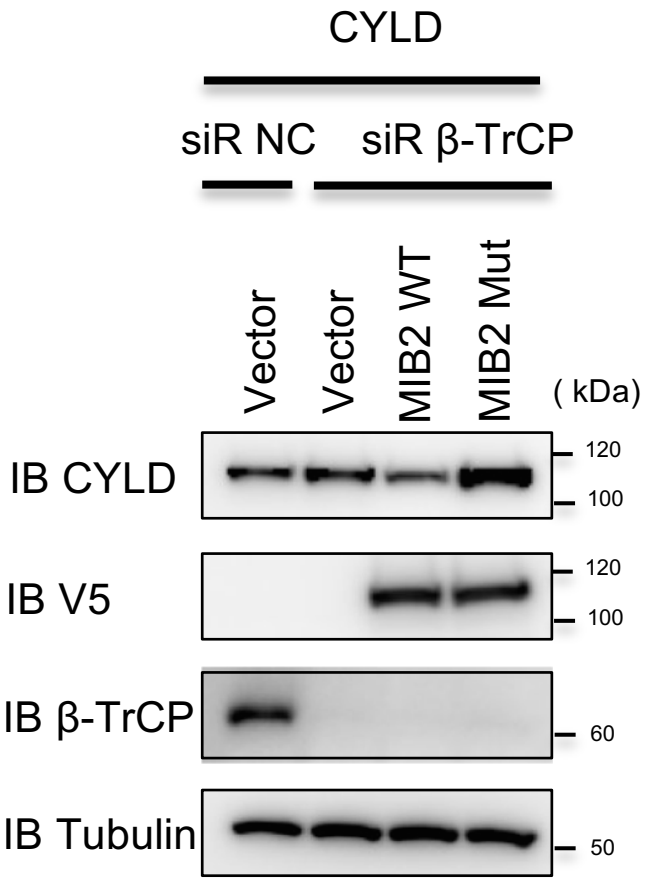

Figure S4. **MIB2 degrades CYLD independently of β-TrCP.** HeLa cells were transfected with an siRNA targeting β-TrCP. Twenty-four hours after transfection, HeLa cells were further transfected with FLAG-tagged CYLD along with V5-tagged WT or Mut MIB2. Control (Vector) was used mock pcDNA3.2 plasmid.

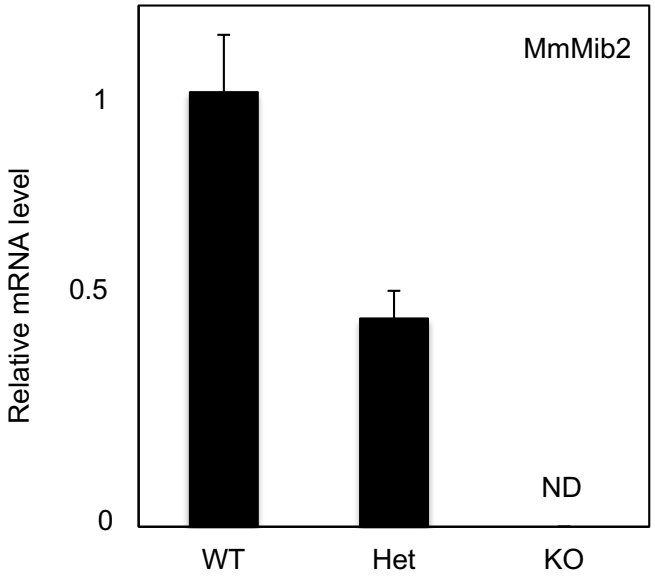

Figure S5. **qPCR analysis of *Mib2* expression in MEF cells.** WT, Het, and KO refer to wild-type, heterozygous and homozygous for the *Mib2* knockout mice, respectively. Relative mRNA levels of *Mib2* are shown by mean ± s.e.m. (*n*=3). ND: not detected.

CYLD mis-sense no.	Codon changes	AA changes	Codon no.	Phenotypes
-	TCA-TAA	Ser-Term	371	Cylindromatosis
-	GAA-TAA	Glu-Term	379	Cylindromatosis
-	CAG-TAG	Gln-Term	403	Cylindromatosis
-	TAT-TAA	Tyr-Term	485	Cylindromatosis
-	TAT-TAG	Tyr-Term	485	Brooke-Spiegler syndrome
-	TAT-TAG	Tyr-Term	523	Cylindromatosis
-	TTA-TAA	Leu-Term	561	Trichoepithelioma
1	GGT-GAT	Gly-Asp	596	Trichoepithelioma
2	GTG-GAG	Val-Glu	654	Brooke-Spiegler syndrome
3	GAT-GGT	Asp-Gly	681	Trichoepithelioma
4	AGA-ACA	Arg-Thr	703	Brooke-Spiegler syndrome
-	CAA-TAA	Gln-Term	707	Brooke-Spiegler syndrome
-	CAA-TAA	Gln-Term	710	Trichoepithelioma
5	GAG-GGG	Glu-Gly	747	Brooke-Spiegler syndrome

CYLD mis-sense no.	Codon changes	AA changes	Codon no.	Phenotypes
-	CGA-TGA	Arg-Term	758	Cylindromatosis
-	AAA-TAA	Lys-Term	767	Brooke-Spiegler syndrome
6	GAT-AAT	Asp-Asn	779	Trichoepithelioma
-	TTA-TGA	Lue-Term	780	Trichoepithelioma
-	TAC-TAG	Tyr-Term	803	Trichoepithelioma
-	CAA-TAA	Gln-Term	823	Cylindromatosis
-	TTA-TAA	Trp-Term	849	Cylindromatosis
-	TGG-TAG	Gln-Term	857	Cylindromatosis
-	GAA-TAA	Glu-Term	868	Cylindromatosis
-	TGG-TGA	Trp-Term	885	Cylindromatosis
7	GGT-GCT	Gly-Ala	896	Trichoepithelioma
8	CCT-CTT	Pro-Leu	904	Trichoepithelioma
-	CGA-TGA	Arg-Term	936	Cylindromatosis
9	GAT-GTT	Asp-Val	941	Trichoepithelioma

Figure S6. Nineteen nonsense and 9 missense mutations in *CYLD* related to Brooke-Spiegler Syndrome, familial cylindromatosis and multiple familial trichoepitheliomas.

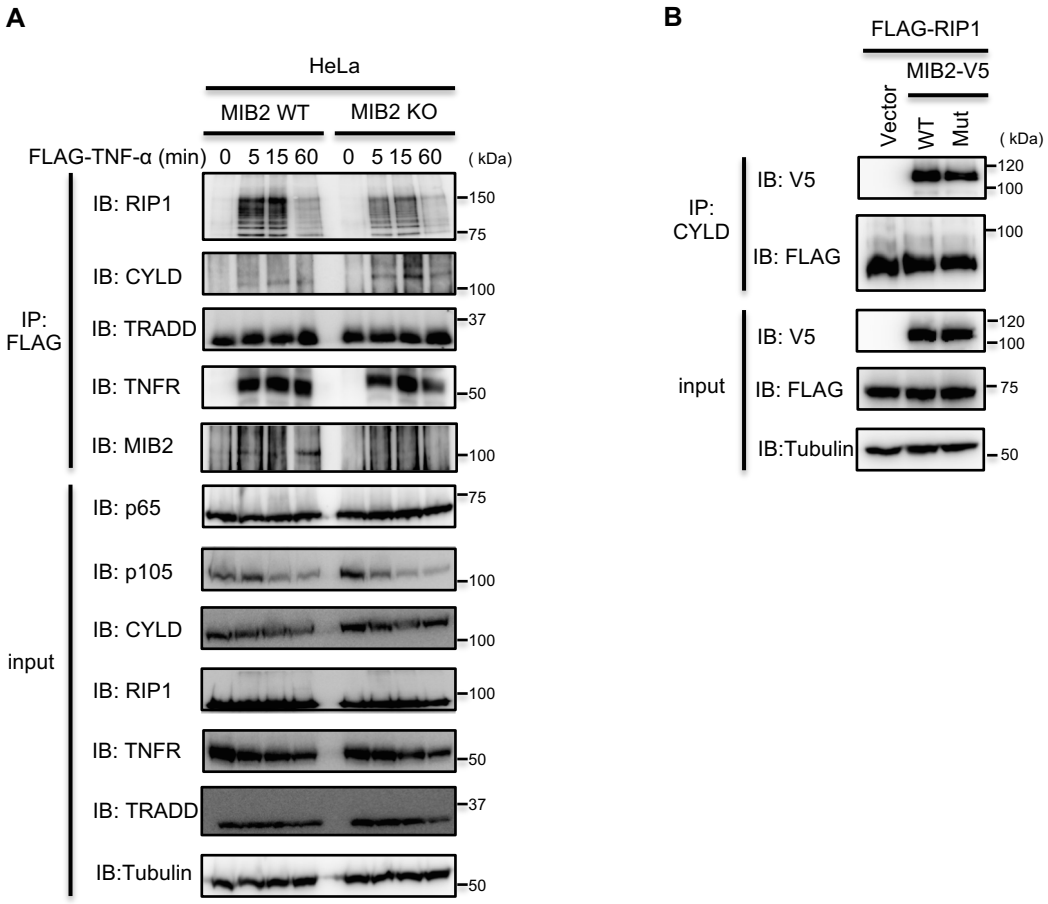

Figure S7. **MIB2 and CYLD are recruited to TNFR signaling complex I.** (A) Analysis of TNF-RSC in *MIB2*-deficient cells. Wild-type (*MIB2*-WT) and *MIB2*-deficient (*MIB2*-KO) HeLa cells were stimulated with FLAG-tagged TNF-α for the indicated times. Following this, the TNF-RSC was examined by immunoprecipitation with an anti-FLAG antibody and immunoblotting of the immunoprecipitates with the indicated antibodies. (B) Analysis of RIP1-MIB2 interaction in cells. Immunoprecipitation using an anti-CYLD antibody was performed on extracts of HEK293T cells transfected with FLAG-tagged RIP1 and either V5-tagged WT or Mut MIB2. Each protein in the immunoprecipitate was evaluated by immunoblotting with the respective antibodies. Control (Vector) was used mock pcDNA3.2.