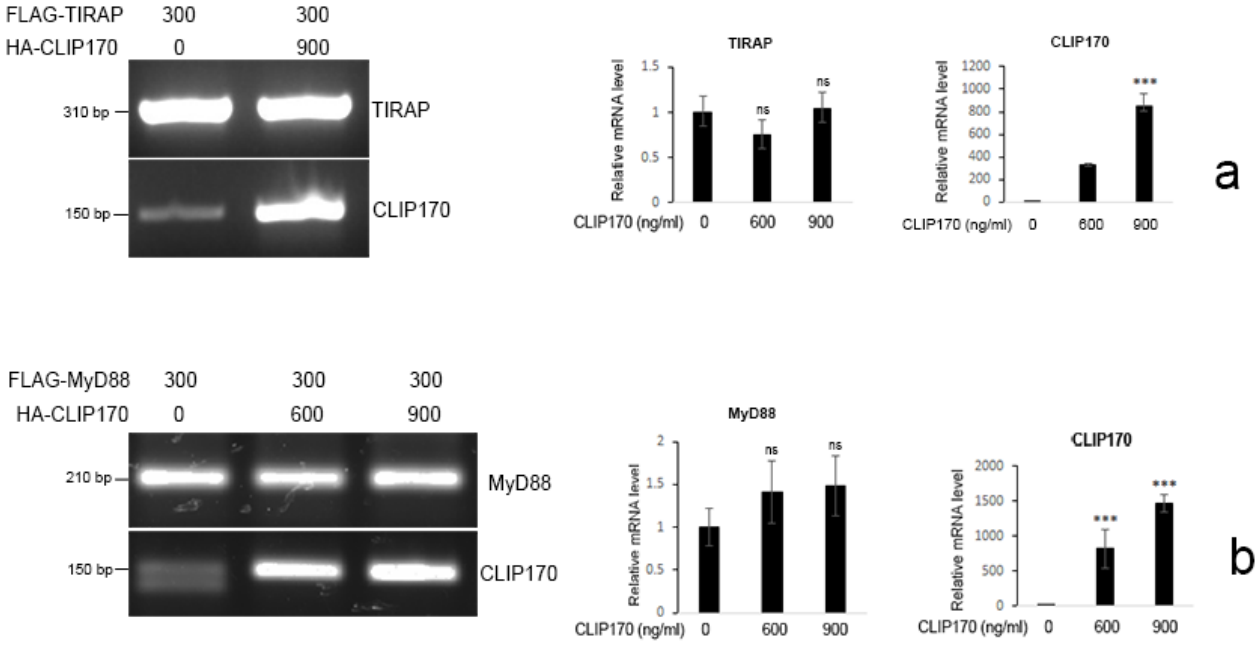
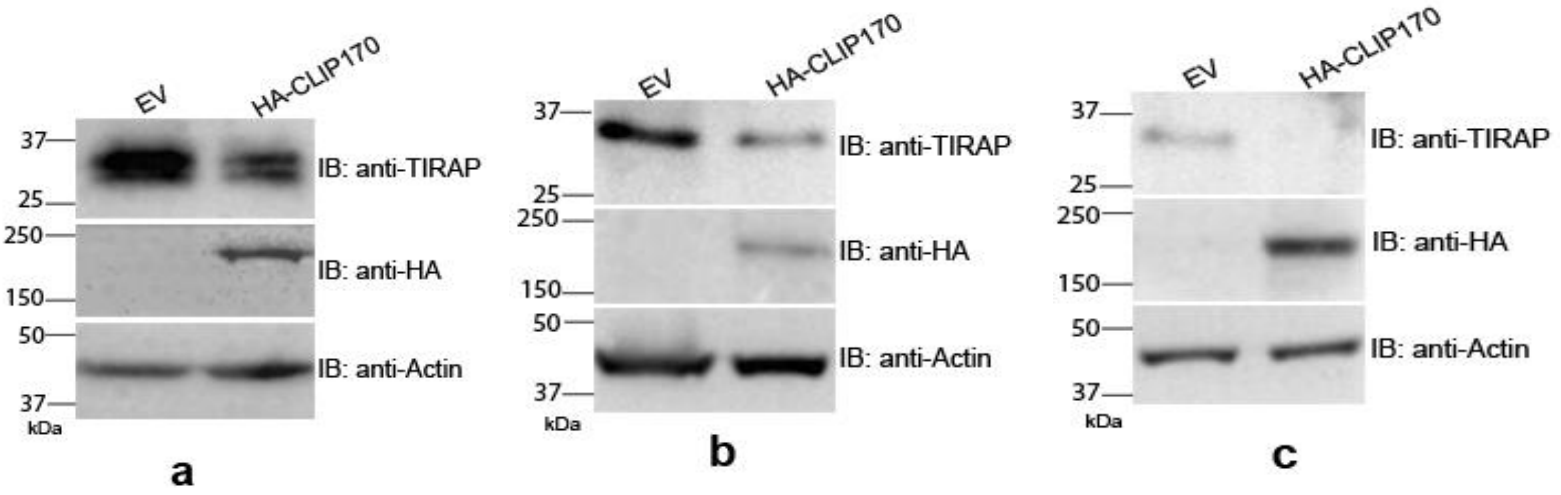
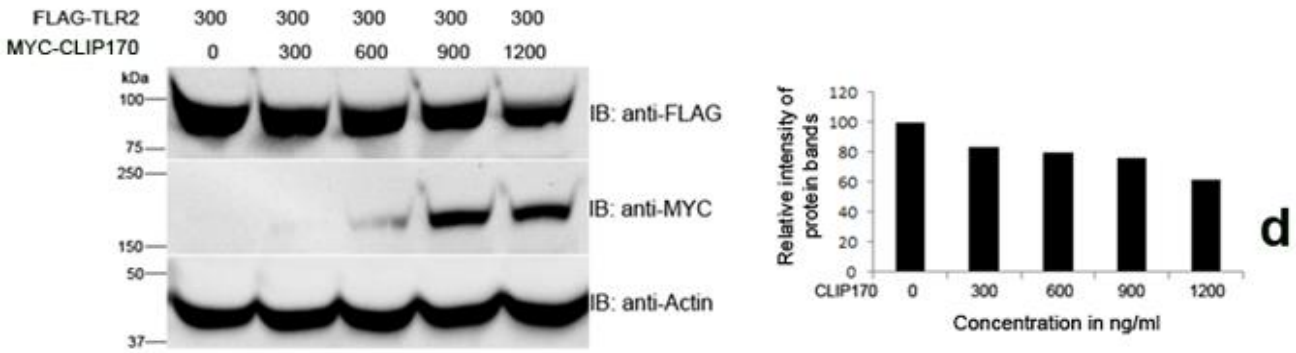
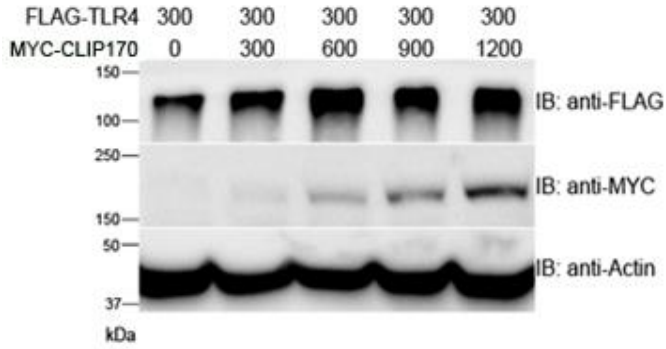


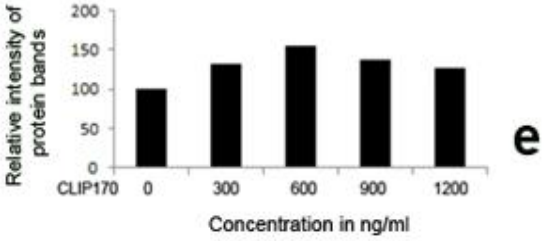
Supplementary Figure 1. Analysis of mRNA levels of TIRAP and MyD88 in the presence of CLIP170. (a) Transcript levels of TIRAP in the presence of CLIP170. HEK293T cells were transfected with FLAG-TIRAP (300 ng/ml) and CLIP170 (0, 600 and 900 ng/ml). Twenty four hours after the transfections, total RNA was extracted from the cells followed by cDNA synthesis. Left panel shows the PCR amplification of a fragment of TIRAP and CLIP170 from the co-transfected samples. Transcript level of TIRAP was not altered in the presence of CLIP170. Right panel shows qPCR analysis of TIRAP or CLIP170 transcript levels in the co-transfected cells. **(b) Transcript levels of MyD88 in the presence of CLIP170**. Reverse transcriptase and qPCR analysis was performed with cells co-transfected with FLAG-MyD88 and CLIP170 as described before. CLIP170 did not alter the transcript level of MyD88. Data are presented as mean ± SD from at least three independent experiments (***$P < 0.001$); ns: not significant.

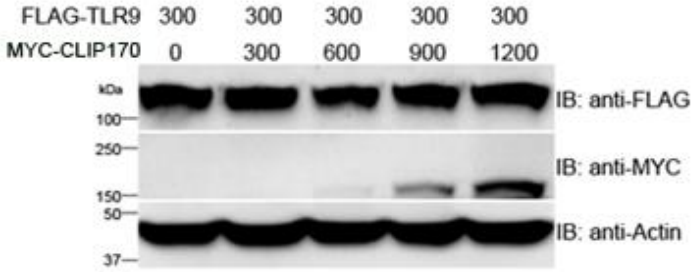

EV
HA-CLIP170
37
25
250
150
50
37
kDa
IB: anti-TIRAP
IB: anti-HA
IB: anti-Actin
a
b
c

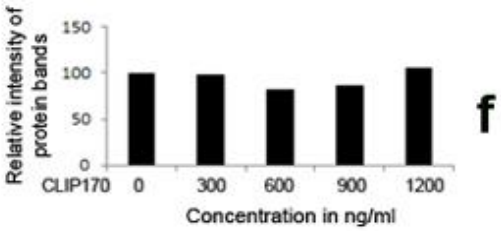

FLAG-TLR2
MYC-CLIP170
300 300 300 300 300
0 300 600 900 1200
kDa
100
75
250
150
50
37
IB: anti-FLAG
IB: anti-MYC
IB: anti-Actin

Relative intensity of protein bands
120
100
80
60
40
20
0
CLIP170 0 300 600 900 1200
Concentration in ng/ml
d

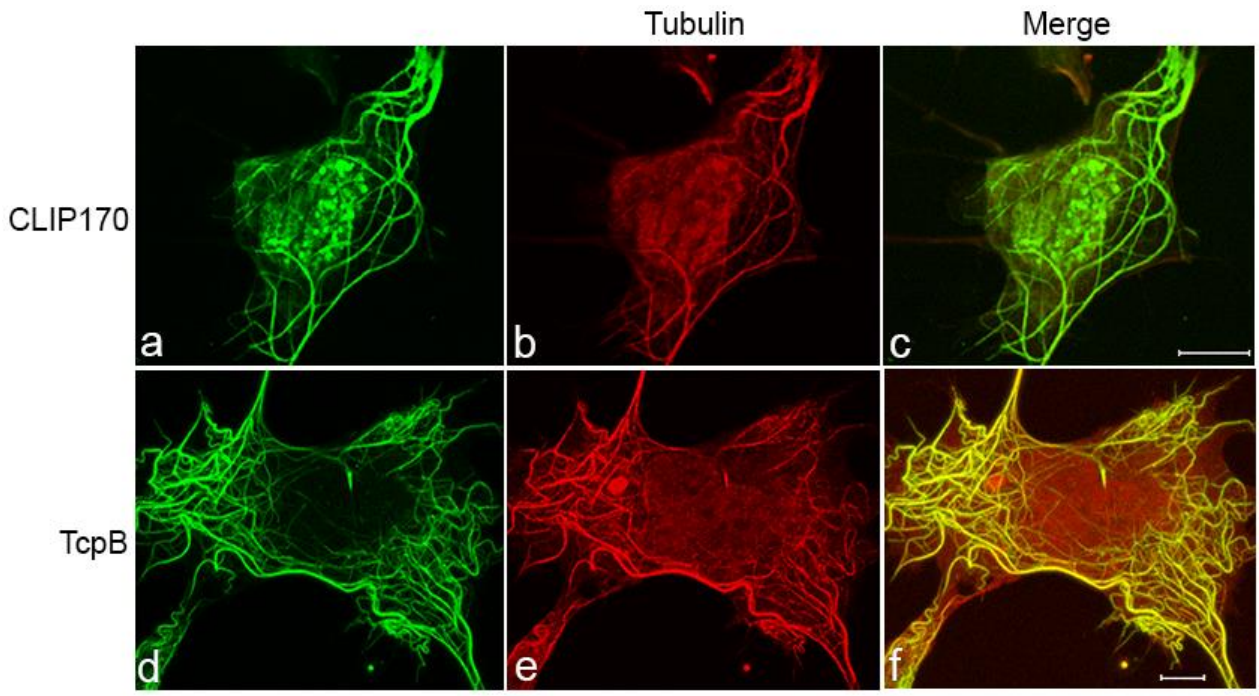

FLAG-TLR4
MYC-CLIP170
300 300 300 300 300
0 300 600 900 1200
150
100
250
150
50
37
kDa
IB: anti-FLAG
IB: anti-MYC
IB: anti-Actin


Relative intensity of protein bands
200
150
100
50
0
CLIP170 0 300 600 900 1200
Concentration in ng/ml
e

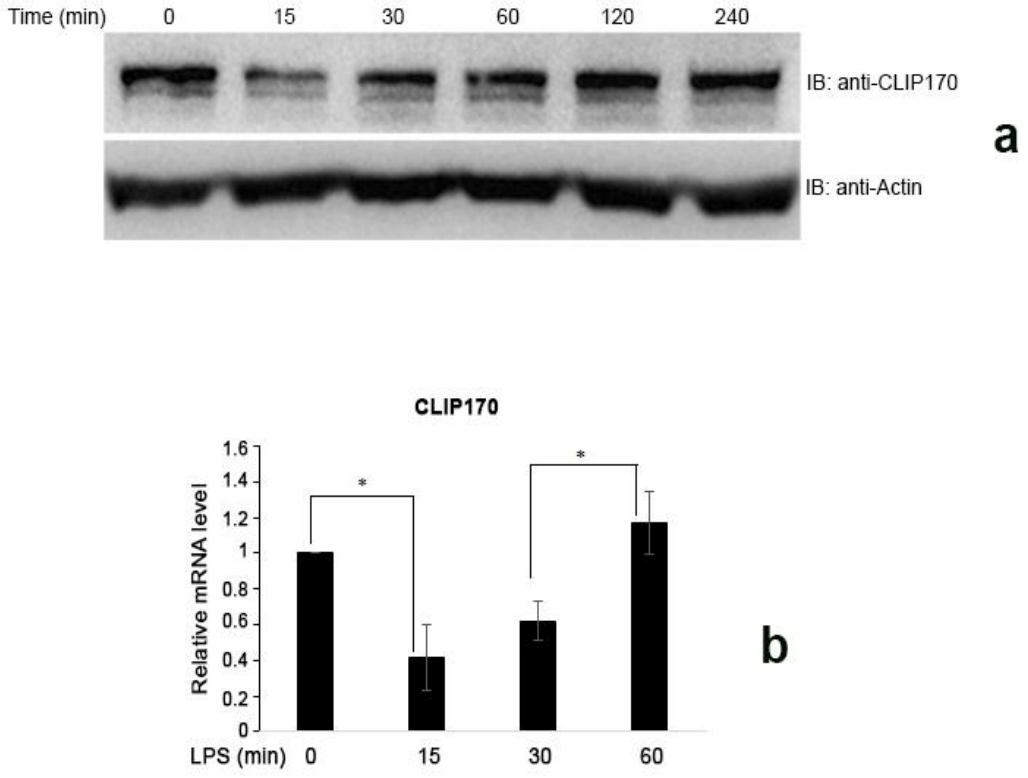

FLAG-TLR9
MYC-CLIP170
300 300 300 300 300
0 300 600 900 1200
kDa
100
250
150
50
37
IB: anti-FLAG
IB: anti-MYC
IB: anti-Actin


Relative intensity of protein bands
150
100
50
0
CLIP170 0 300 600 900 1200
Concentration in ng/ml
f

Supplementary Figure 2. (a-c) CLIP170 induces degradation of endogenous TIRAP. RAW264 cells were transfected with EV or HA-CLIP170 followed by detection of endogenous TIRAP using anti-TIRAP antibodies from Cell Signaling Technology (CST, # 13077S, Suppl. Fig. 2a), Santa Cruz Biotechnology (# sc-166149, Suppl. Fig. 2b) and Abcam (# ab17218, Suppl. Fig. 2c). Anti-rabbit IgG, HRP-linked secondary antibody (CST # 7074) was used for blots probed with anti-TIRAP antibody from CST and Abcam. Anti-mouse IgG, HRP-linked secondary antibody (CST # 7076) was used for the blot probed with anti-TIRAP antibody from Santa Cruz Biotechnology. HA-CLIP170 was detected using HRP conjugated anti-HA antibody (Sigma # H6533). Actin was detected using peroxidase-conjugated anti-β-actin monoclonal antibody (Sigma # A3854). Actin served as the loading control**. (d-f) Effect of CLIP170 on TLR2, TLR4 and TLR9**. HEK293T cells were co-transfected with FLAG-tagged TLR2, TLR4, or TLR9, and increasing concentrations of MYC-CLIP170. Twenty four hours post-transfection, cells were lysed and subjected to immunoblotting. The blot was probed with anti-FLAG and anti-MYC antibodies to detect FLAG-tagged TLRs and MYC-CLIP170, respectively. Actin served as the loading control. Right panel of the immunoblot shows the densitometry analysis of FLAG-TLR bands normalized to actin. CLIP170 induced slight degradation of TLR2 (d). TLR4 underwent slightly enhanced ubiquitination in the presence of CLIP170 (e) whereas levels of TLR9 was not affected by CLIP170 (f). Immunoblots are representative of two independent experiments.



Supplementary Figure 3. Sub-cellular localization of CLIP170 or TcpB in Mouse Embryonic Fibroblast cell lines. MEF cells were transfected with wild-type MYC-CLIP170, or HA-TcpB. Twenty four hours post-transfection, cells were fixed, permeabilized, and stained with FITC labelled anti-MYC antibody to detect CLIP170 (a-c) or FITC conjugated anti-HA antibody to detect HA-TcpB (d-f). Tubulin was stained with Cy3 labelled anti-tubulin antibody. HA-CLIP170 co-localized with microtubules and induced microtubule bundling with varying intensity in 85% of the MEF cells that were analysed. HA-TcpB co-localized and induced severe microtubule bundling in 100% of MEF cells that were analysed. Scale bar indicates 10 µm.



Supplementary Figure 4. LPS modulates the expression of CLIP170 in mouse macrophages. (a) Immunoblot analysis of LPS-challenged J774 cells. J774 cells were challenged with LPS for the indicated time points followed by immunoblotting. The blot was probed with anti-CLIP170 to examine the endogenous levels of CLIP170 protein. Actin served as the loading control. Immunoblots are representative of three independent experiments. **(b) Quantitative PCR analysis of CLIP170 expression in LPS challenged J774 cells.** J774 cells were challenged with LPS followed by RNA isolation and qPCR analysis. Data are presented as mean ± SD from three independent experiments; **P* < 0.05.