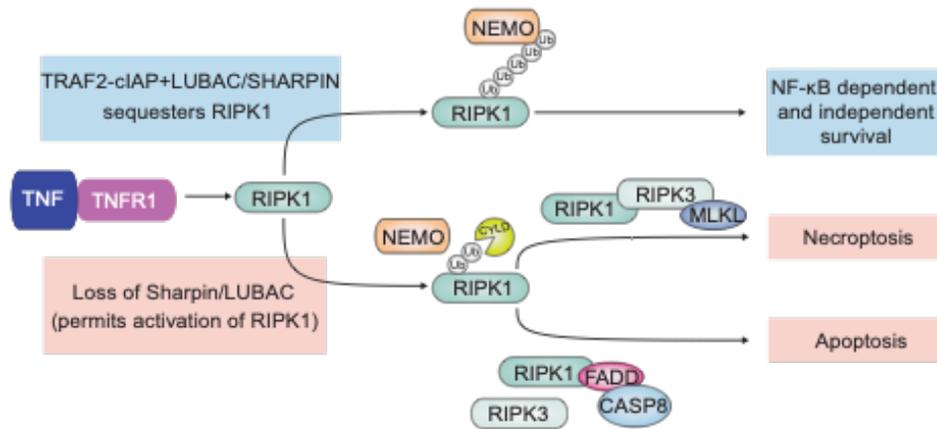


## Supplemental Figures:

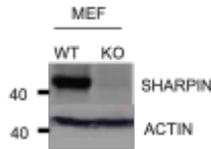
Supplemental Figure 1



### Supplemental Fig 1: Schematic overview of TNF/TNFR induced signaling pathways.

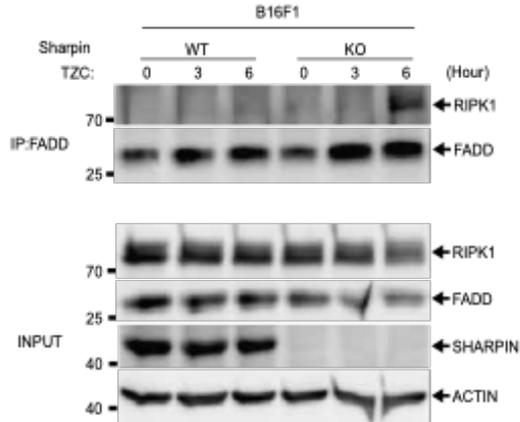
Ubiquitination of RIPK1 catalyzed by TRAF2/cIAP1/2 and the LUBAC (containing SHARPIN) E3 ligases to conjugate lysine 63 (K63) and linear (M1) poly-ubiquitination, respectively, stabilizes the RIPK1's interaction with NEMO, a component of the IKK complex. RIPK1-NEMO association inhibits RIPK1 from interacting with the death-signaling Complex II containing FADD, CASPASE 8 and RIPK3, and permits the phosphorylation of RIPK1 by IKK family kinases. In the absence/dysfunction of the E3 ligases and/or the upregulation of deubiquitinases, RIPK1 interacts with FADD and CASPASE 8 to initiate downstream activation of CASPASE 8 and trigger apoptosis. RIPK3 is a non-essential enhancer of apoptosis via this pathway (1, 2). If CASPASE 8 activity is inhibited, deubiquitinated RIPK1 induces activation of RIPK3 and ultimately activates mixed lineage kinase domain-like protein (MLKL)-dependent necroptosis.

## Supplemental Figure 2



**Supplemental Figure 2:** Immunoblot of WT or SHARPIN knockout (SHARPIN KO) murine embryonic fibroblasts (representative of 2 independent experiments).

## Supplemental Figure 3



**Supplemental Figure 3.** Activation of apoptosis pathway and recruitment of RIPK1 to DISC in SHARPIN KO but not WT B16F1 cells. B16F1 cells of both genotypes were stimulated with TNF (100 ng/ml) in the presence of zVAD-FMK (20  $\mu$ M) and cycloheximide (1  $\mu$ g/ml) for 0, 3 and 6 hours. Lysates from these cells were immunoprecipitated with anti-FADD to isolate the death-signaling Complex II, followed by western blotting with anti-RIPK1. Prior to immunoprecipitations, aliquots of the total lysates were reserved (input) and analyzed by western blotting with the indicated antibodies.

## References:

1. Dondelinger Y, Aguilera MA, Goossens V, Dubuisson C, Grootjans S, Dejardin E, et al. RIPK3 contributes to TNFR1-mediated RIPK1 kinase-dependent apoptosis in conditions of cIAP1/2 depletion or TAK1 kinase inhibition. *Cell Death Differ.* 2013;20(10):1381-92.
2. Lalaoui N, Boyden SE, Oda H, Wood GM, Stone DL, Chau D, et al. Mutations that prevent caspase cleavage of RIPK1 cause autoinflammatory disease. *Nature.* 2020;577(7788):103-8.