

Supplemental Figures and Figure Legends

Supplementary Figure S1. *Rip1*^{K45A/K45A} or *Rip1*^{Δ/Δ} mice are viable and healthy.

(a) Sequencing results of *Rip1*^{K45A/K45A} mice. (b) Schematic map of RIP1 and sequencing results of *Rip1*^{Δ/Δ} mice. (c) Representative photographs of 8-week-old WT and *Rip1*^{Δ/Δ} mice. (d) RIP1 protein levels in different tissues including thymus, lung, liver and spleen from WT, *Rip1*^{K45A/K45A} and *Rip1*^{Δ/Δ} mice.

Supplementary Figure S2. *Rip1*^{Δ/Δ} mice show normal B/T cells development.

(a) Flow cytometric analysis of the cells isolated from thymus, lymph nodes, and spleen of 8 weeks *Rip1*^{Δ/Δ} mouse and littermate WT control mouse. (b) Absolute organ cellularity of thymus, lymph nodes, and spleen from 8 weeks *Rip1*^{Δ/Δ} mice (n=3) and littermate WT control mice (n=3). Error bars represent mean ± SD.

Supplementary Figure S3. RIP1^Δ interacts well with RIP3.

Immunoprecipitation of RIP1^Δ with RIP3 in 293T cells, wild-type of RIP1 as positive control.

Supplementary Figure S4. RIP1^Δ does not influence NF-κB and MAPK signaling pathways.

BMDMs from WT and *Rip1*^{Δ/Δ} mice were treated with LPS (100ng/ml) for different time periods as indicated, and the lysates were analyzed by western blot for p-IκB, p-JNK, JNK, p-P38, P38, p-P65, ERK, p-ERK, RIP1 and beta-actin.

Supplementary Figure S5. RIP1^Δ as well as RIP1^{K45A} block apoptosis induced by TNF/Smac.

(a) MDFs from wild-type, *Rip1*^{-/-}, *Rip1*^{K45A/K45A} and *Rip1*^{Δ/Δ} mice were treated for 24 hour as indicated with TNF (30ng/ml), CHX (10ug/ml). Cell viability was determined by measuring intracellular ATP levels with a Cell Titer-Glo Luminescent Cell Viability Assay kit. ***P<0.0001 by Student's *t*-test. Data are represented as the mean ± SEM of three independent experiments. (b) MDFs from wild-type, *Rip1*^{-/-},

Rip1^{K45A/K45A} and *Rip1*^{Δ/Δ} mice were treated for 24 hour as indicated with TNF (30ng/ml), Smac mimetic (1uM). Cell viability was determined by measuring intracellular ATP levels with a Cell Titer-Glo Luminescent Cell Viability Assay kit. ***P<0.0001 by Student's *t*-test. Data are represented as the mean ± SEM of three independent experiments. (c) MDFs from wild-type, *Rip1*^{-/-}, *Rip1*^{K45A/K45A} and *Rip1*^{Δ/Δ} mice were treated with DMSO, TC and TS for 24h and lysates were collected and subjected to western blot analysis of cleaved-PARP, cleaved- Caspase-8, Caspase-3 and β-actin.

Supplementary Figure S6. *Rip1*^{Δ/Δ} MEFs resist necroptosis to the same extent as *Rip1* knockout MEFs.

(a) MEFs from wild-type and *Rip1*^{-/-} mice were treated with DMSO, TC, TCZ and TCZN respectively for 24h. Cell viability was determined by measuring ATP levels using the CellTiter-Glo kit. **P<0.0005, ***P<0.0001 by Student's *t*-test. Data are represented as the mean ± SEM of three independent experiments. (b) MEFs from wild-type and *Rip1*^{-/-} mice were treated with DMSO, TCZ and TCZN respectively for 12h. Cell lysate was collected and subjected to western blot analysis of RIP3, p-RIP3, MLKL, p-MLKL and β-actin levels. (c) MEFs from wild-type and *Rip1*^{Δ/Δ} mice were treated with DMSO, TSZ, TSZN, TC, TCZ and TCZN respectively for 24h. Cell viability was determined by measuring intracellular ATP levels with a Cell Titer-Glo Luminescent Cell Viability Assay kit. ***P<0.0001 by Student's *t*-test. Data are represented as the mean ± SEM of three independent experiments. (d) MEFs from wild-type and *Rip1*^{Δ/Δ} mice were treated with DMSO, TSZ and TSZN respectively for 12h. Cell lysate was collected and subjected to western blot analysis of RIP3, p-RIP3, MLKL, p-MLKL and β-actin levels.

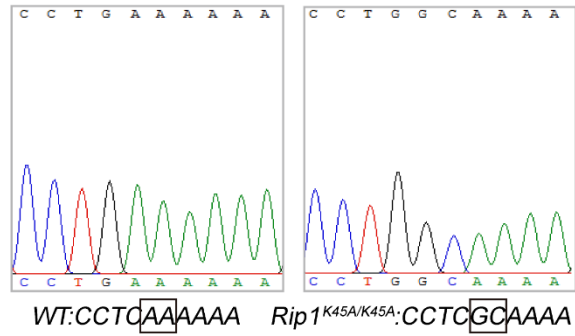
Supplementary Figure S7. *Rip1*^{Δ/Δ} MDFs are more resistant to necroptosis than *Rip1*^{K45A/K45A} cells, but *Fadd*^{-/-}*Rip1*^{Δ/Δ} MEFs undergo spontaneous cell death.

(a) Microscopic photographs of *Rip1*^{Δ/Δ} and *Fadd*^{-/-}*Rip1*^{Δ/Δ} MEFs. Scale bar, 10μm. Control cells are *Rip1*^{Δ/Δ}. (b) MDFs with Indicated genotypes were treated with

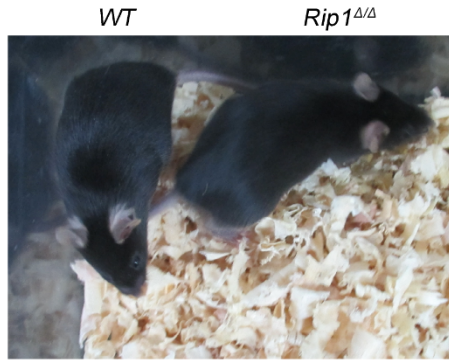
different concentration of TNF- α plus Smac (500nM)/ZVAD respectively for 12 hours. Cell viability was determined by measuring intracellular ATP levels with a Cell Titer-Glo Luminescent Cell Viability Assay kit. *P<0.001 by Student's *t*-test. Data are represented as the mean \pm SEM of three independent experiments.

Supplementary Figure S1

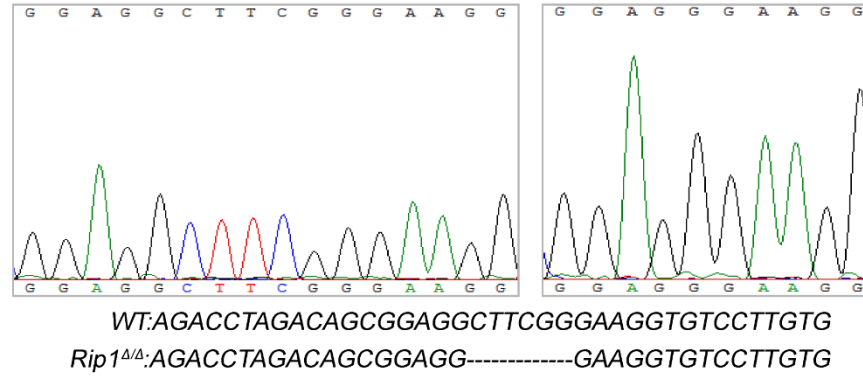
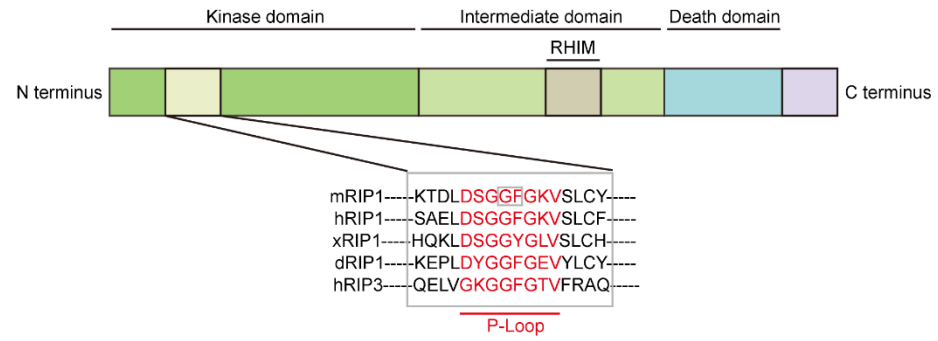
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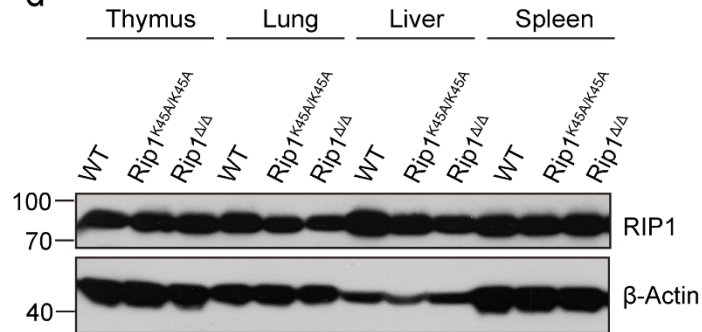
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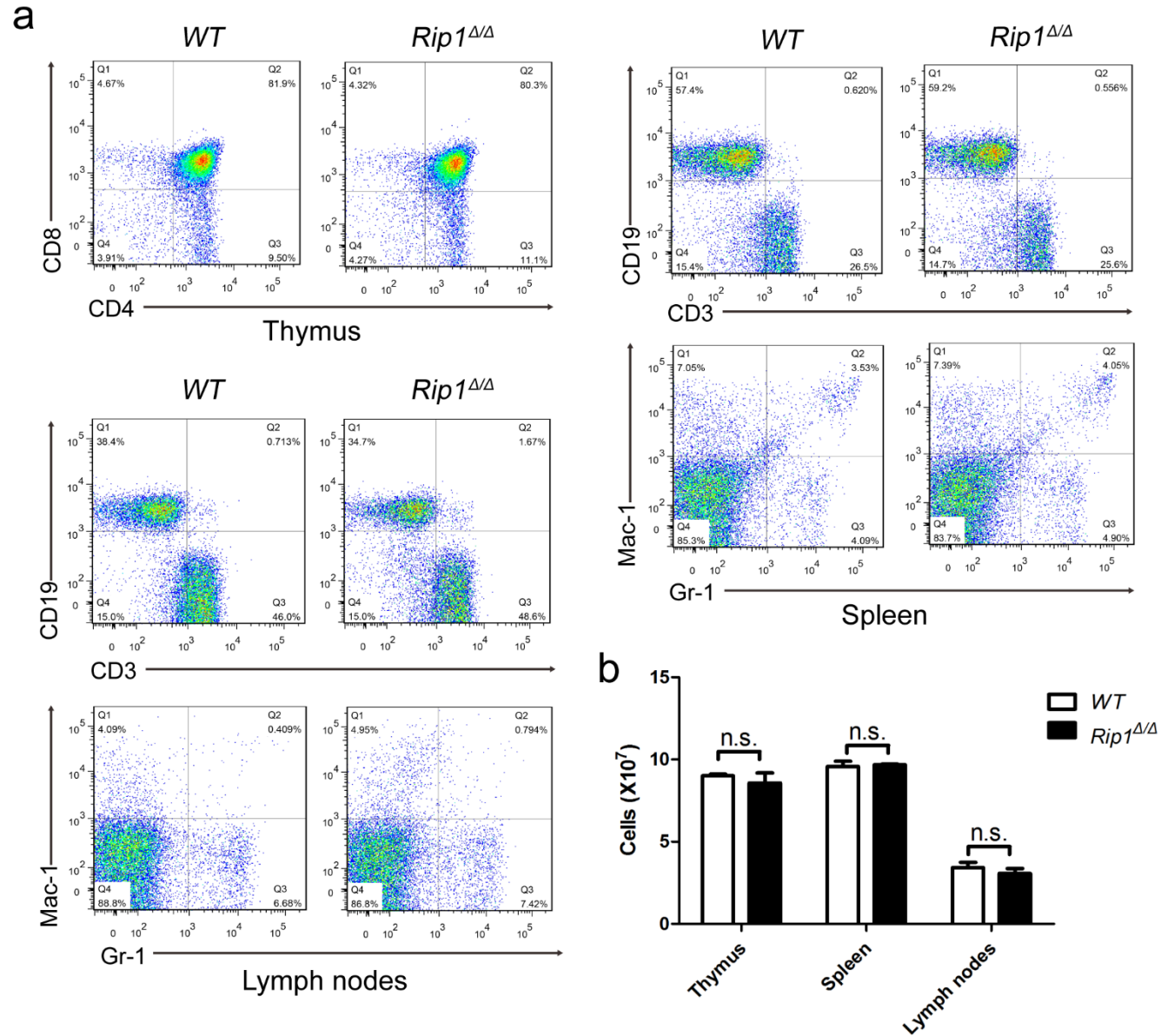
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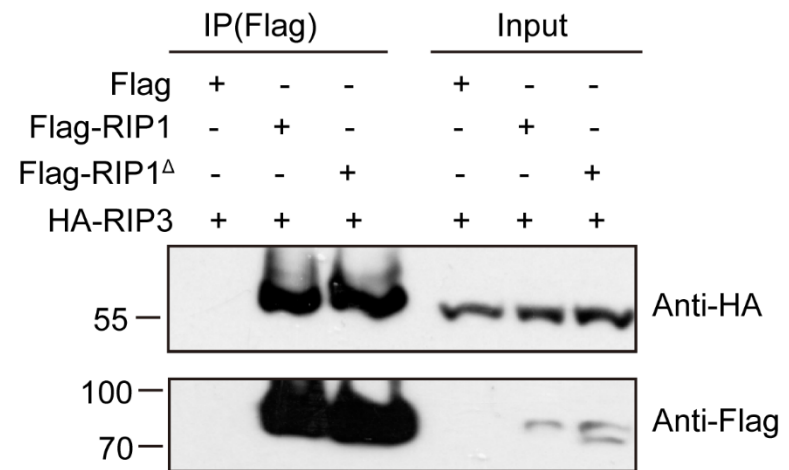
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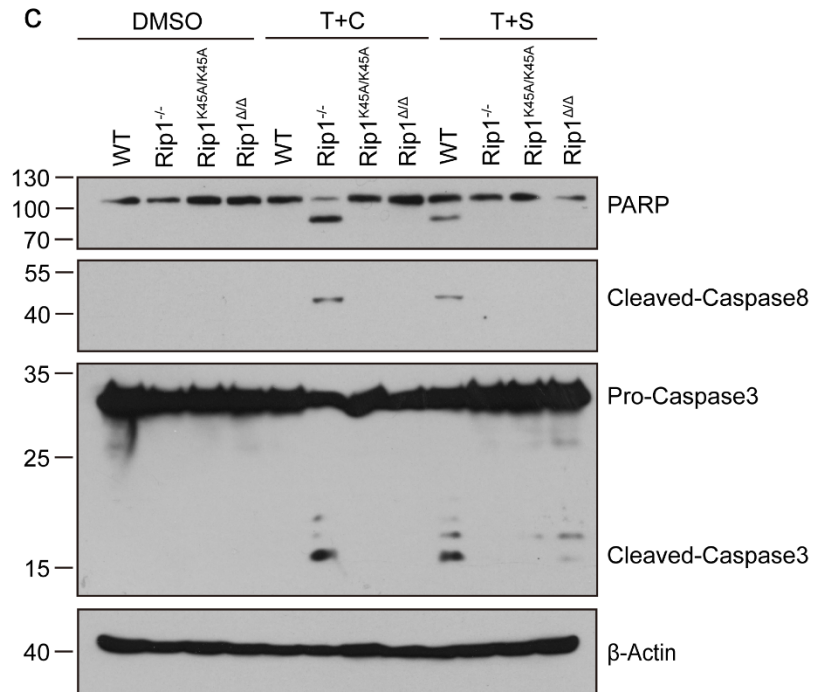
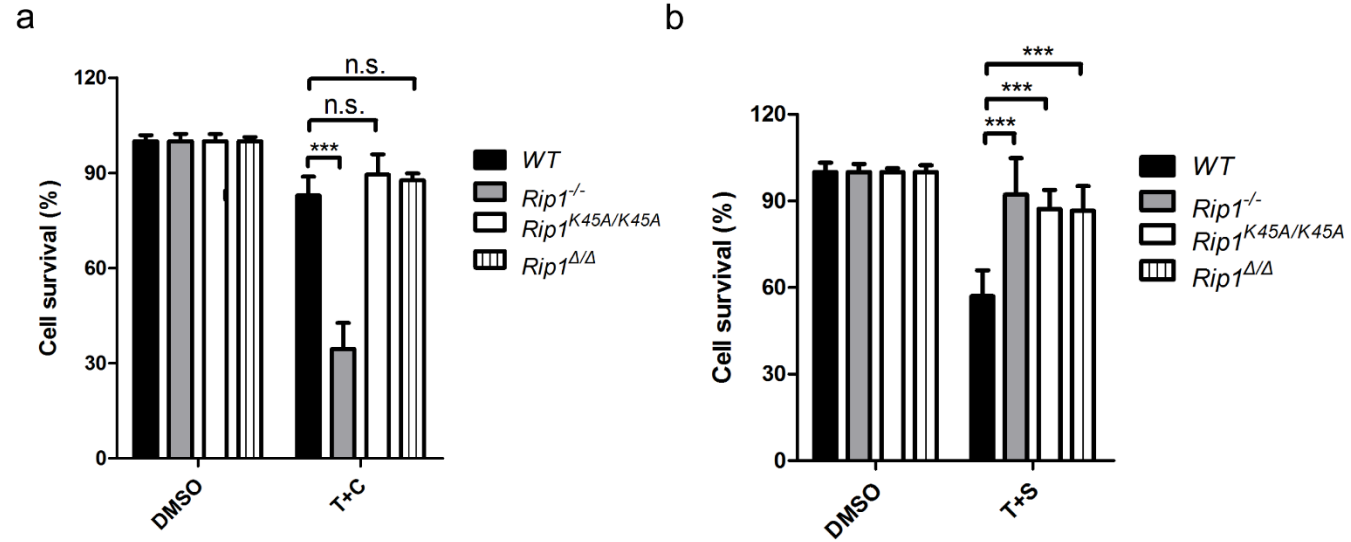
Supplementary Figure S2



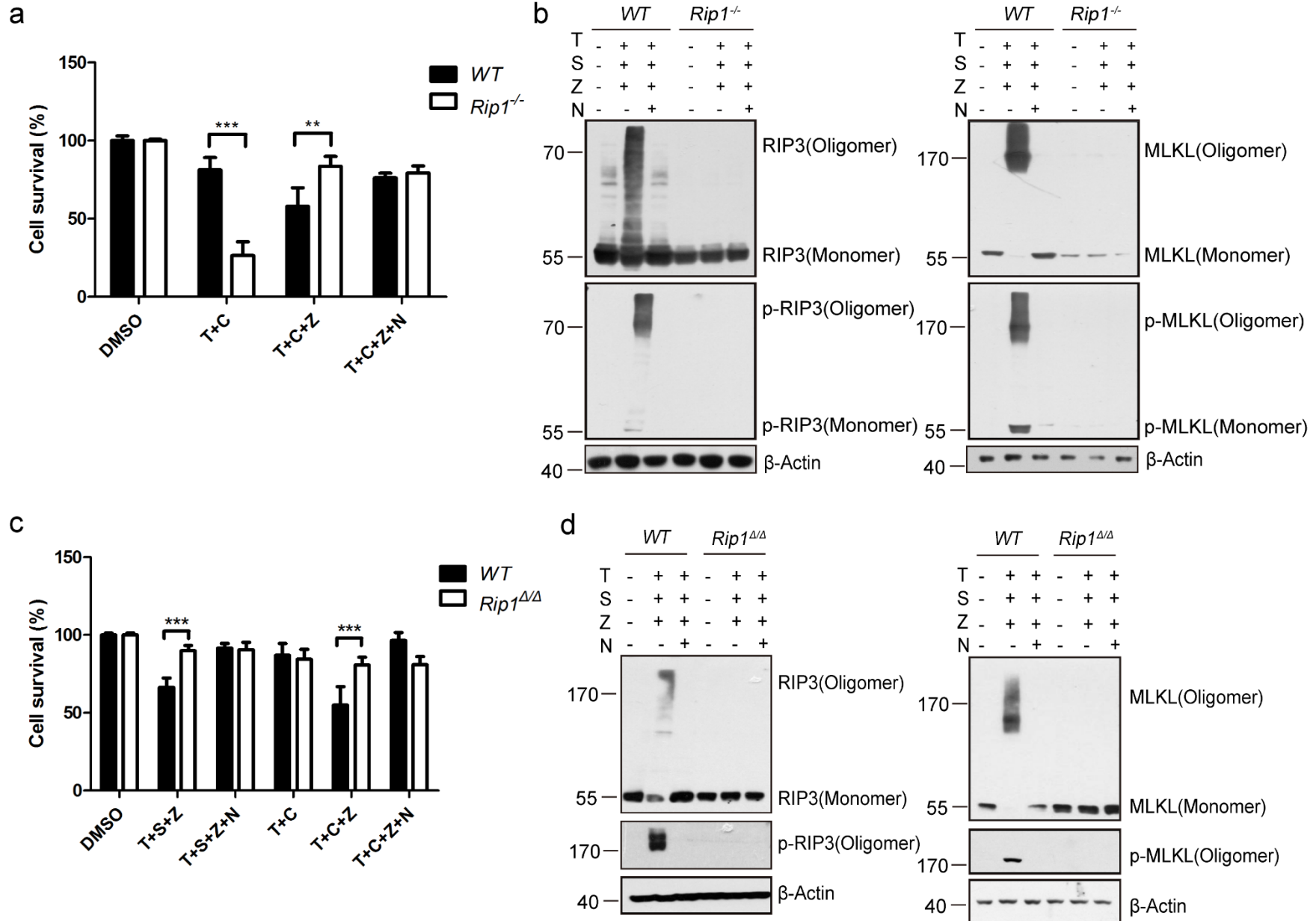
Supplementary Figure S3



Supplementary Figure S5



Supplementary Figure S6



Supplementary Figure S7

