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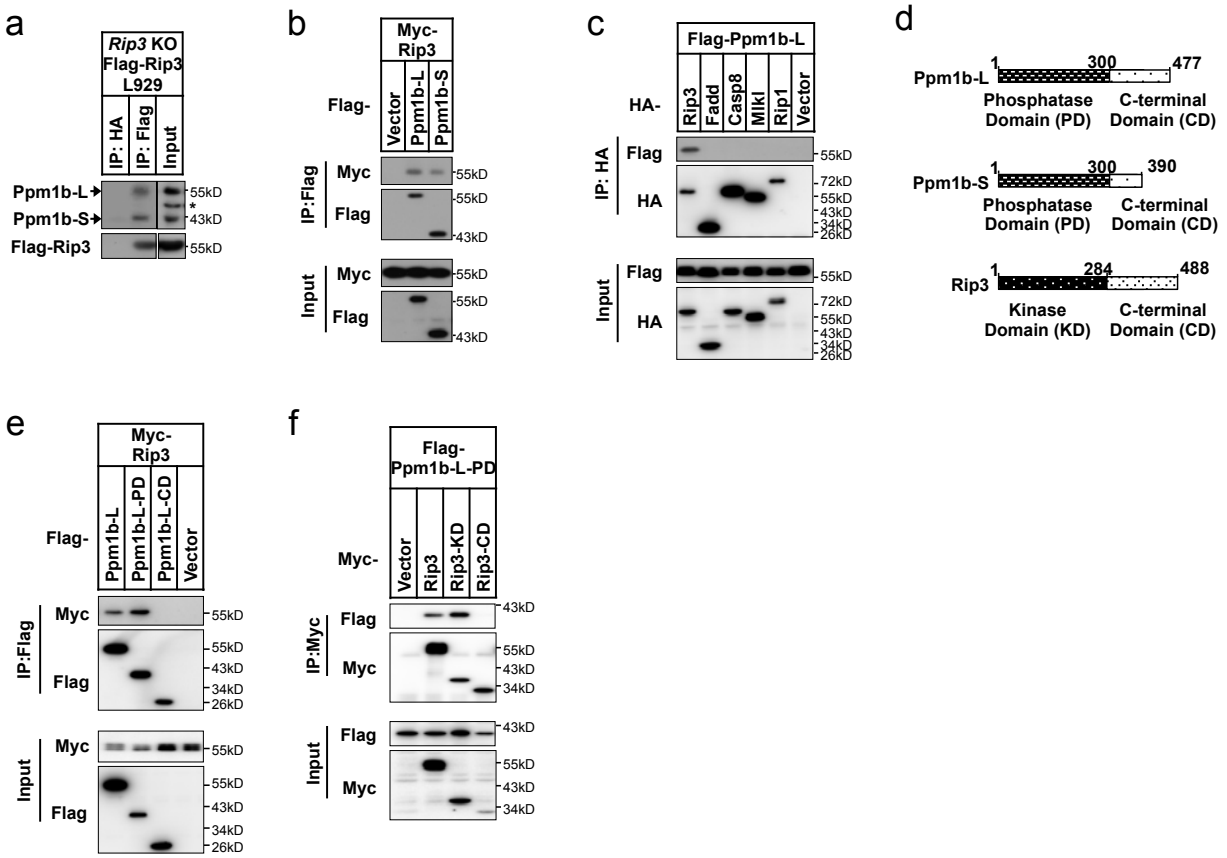
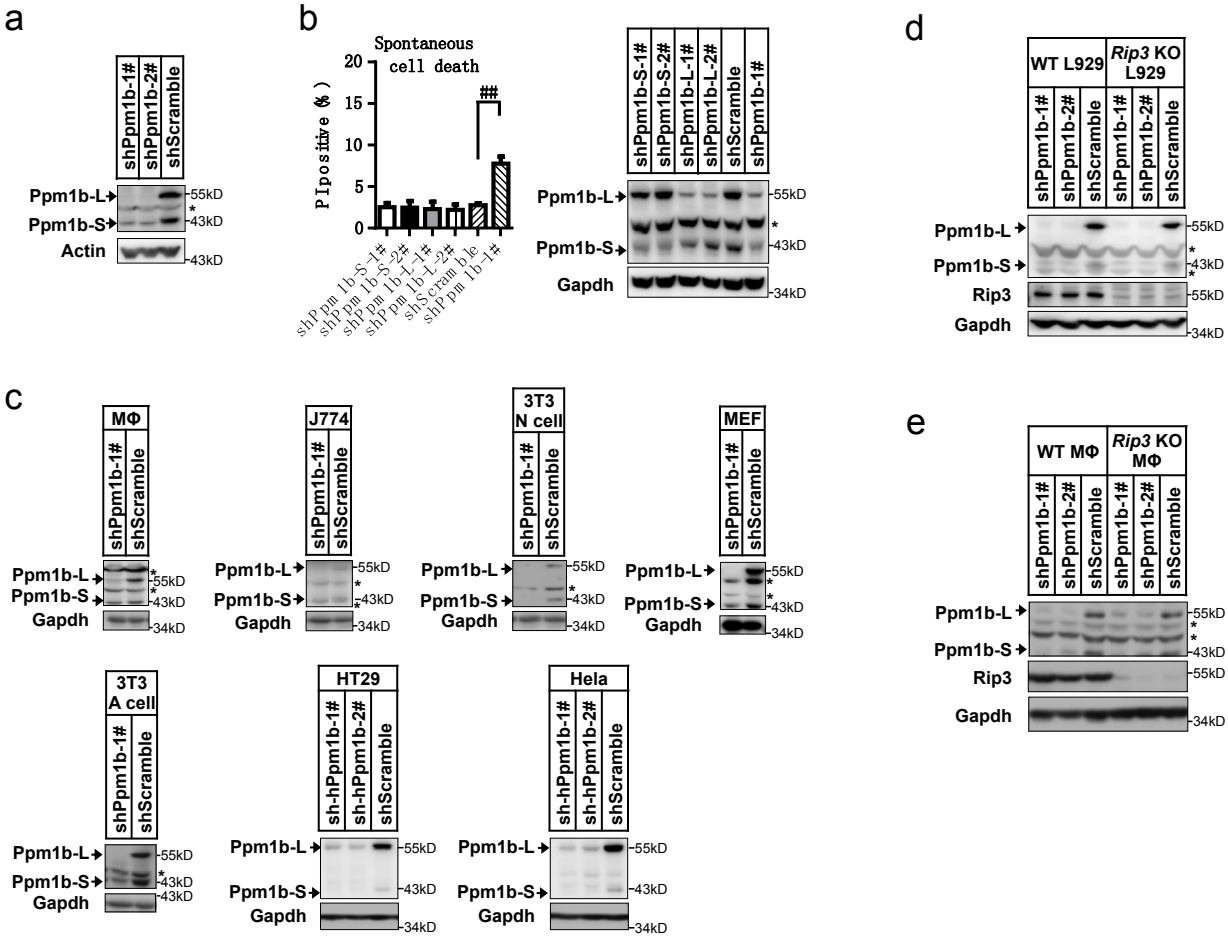
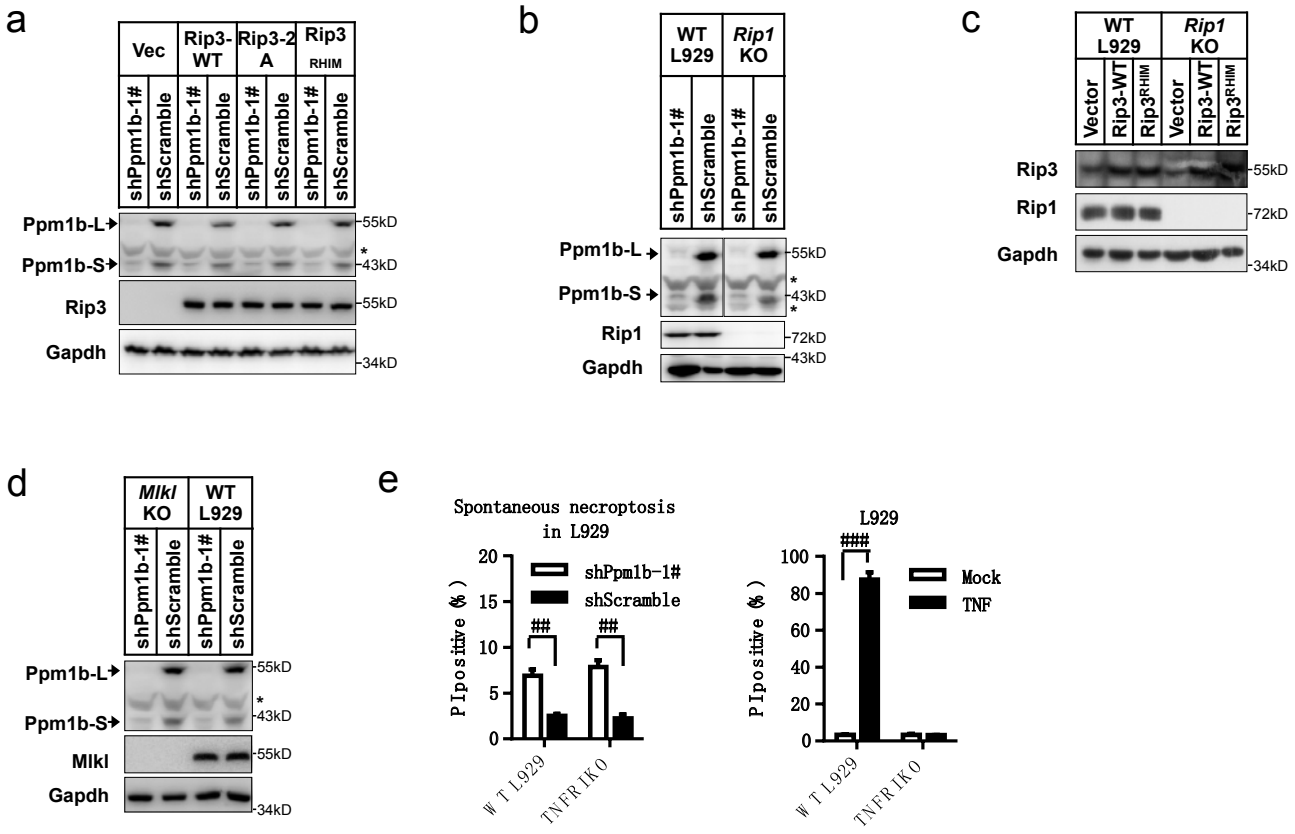


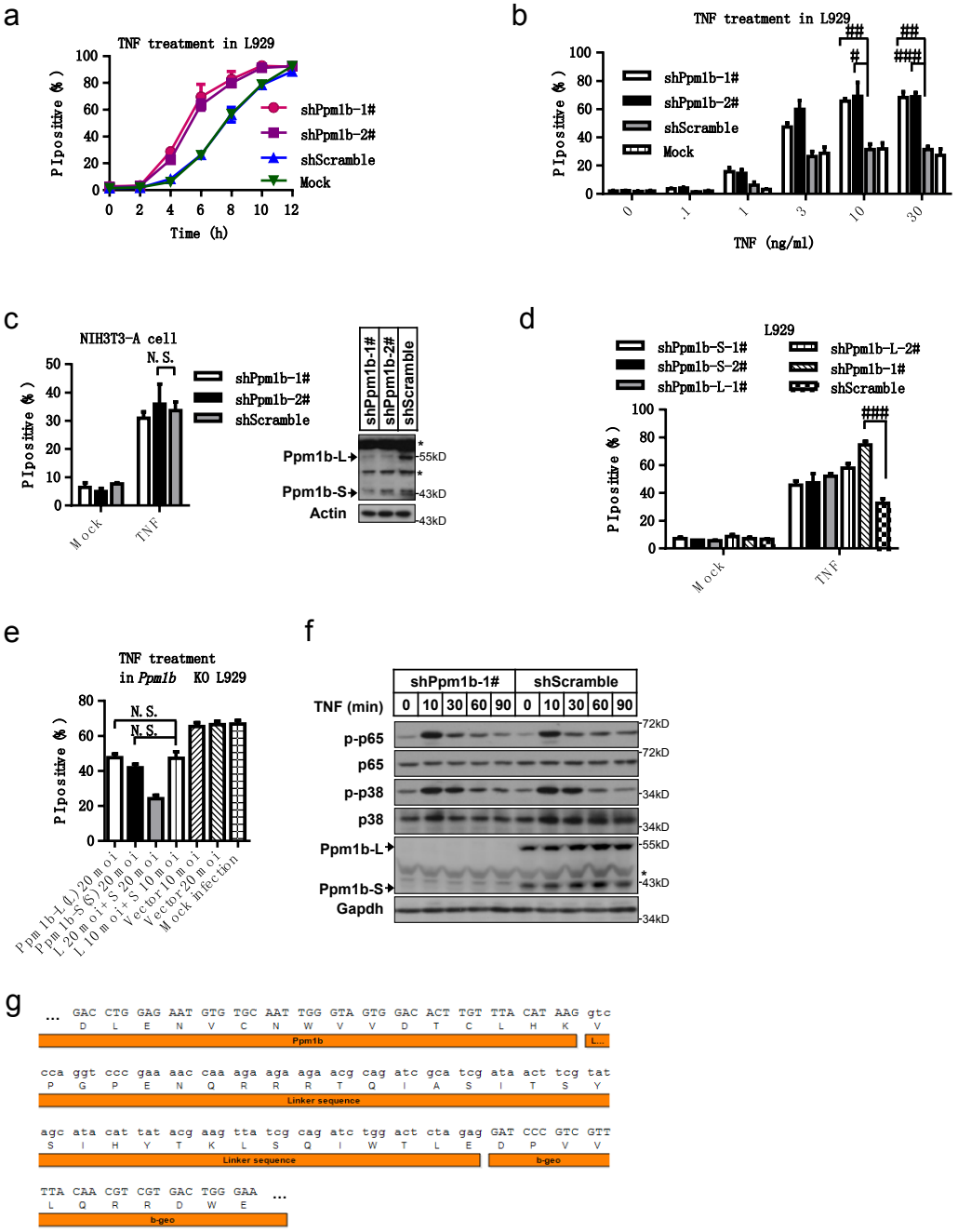
Supplementary Figure 1 Ppm1b interacts with Rip3. (**a**) Ppm1b interacts with Rip3 in resting stage in L929 cells. Rip3-KO-Flag-Rip3 L929 cells were lysed and the cell lysates were immunoprecipitated with anti-HA (IP: HA) and anti-Flag (IP: Flag) antibodies. Both the cell lysate (Input) and immunoprecipitates were analyzed by immunoblotting with indicated antibodies. The vertical line represents a splice mark. The samples were obtained and processed in the same experiment, and the gels/blots were processed in parallel. (**b**) Both Ppm1b-L and Ppm1b-S co-immunoprecipitate with Rip3 in 293T cells. Myc-Rip3 was coexpressed with Flag-tagged Ppm1b-L, Ppm1b-S or empty vector. Then co-immunoprecipitation experiments were performed with anti-Flag antibody. Both the immunoprecipitates and lysates were analyzed by immunoblotting with indicated antibodies. (**c**) Ppm1b selectively interacts with Rip3 but not the other necrosome components in 293T cells. Flag-Ppm1b-L was coexpressed with HA-tagged proteins as indicated. Co-immunoprecipitaton experiments were performed with anti-HA antibody and analyzed as in (b) (**d**) Schematic of the domain structures of Ppm1b-L and Ppm1b-S and Rip3. (**e**) The phosphatase domain of Ppm1b interacts with Rip3. Myc-Rip3 was coexpressed with Flag-tagged full length and truncated Ppm1b in 293T cells. Then co-immunoprecipitation experiments were performed as in (b). Ppm1b-L-PD: Ppm1b-L phosphatase domain; Ppm1b-L-CD: Ppm1b-L C-terminal domain. (**f**) The kinase domain of Rip3 interacts with Ppm1b. Flag-Ppm1b-L-PD was coexpressed with Myc-tagged full length and truncated Rip3 in 293T cells. Then co-immunoprecipitation experiments were performed with anti-Myc antibody and analyzed as in (b). Rip3-KD: Rip3 kinase domain; Rip3-CD: Rip3 C-terminal domain. Data shown were representative of two or more independent experiments. Uncropped images of blots are shown in Supplementary Figure 7.



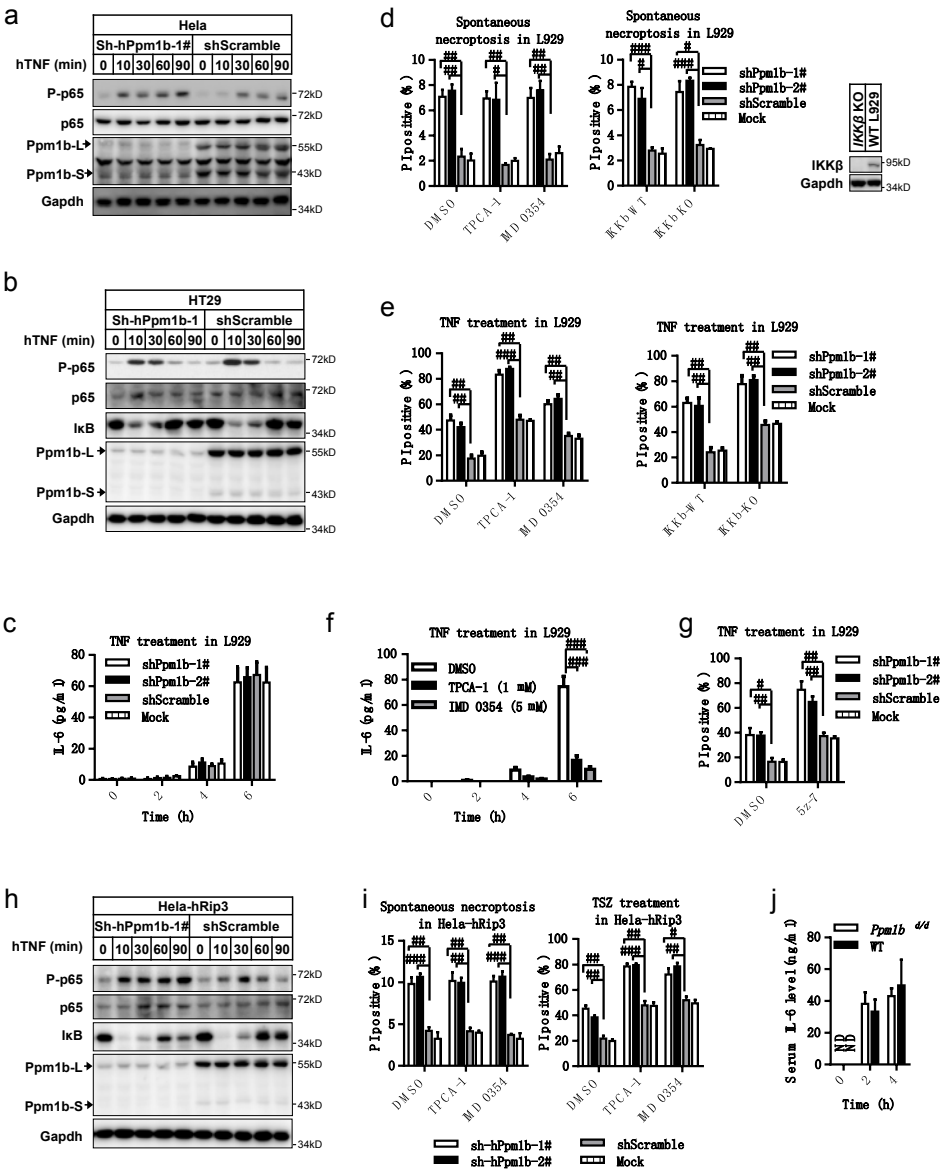
Supplementary Figure 2 Related to Figure 2. (**a**) The protein amounts in Ppm1b knocked down and control L929 cells were analyzed by immunoblotting with indicated antibodies. Related to Figure 2a. (**b**) Ppm1b-S, Ppm1b-L and both isoforms were knocked down with isoform-specific shRNAs in L929 cells. Left, 48 hours later, spontaneous cell death was measured. Right, the protein amounts were analyzed by immunoblotting with indicated antibodies. Results shown were mean ± s.e.m.; n = 3000 cells pooled from 3 independent experiments. (**c**) The protein amounts in Ppm1b knocked down and control cells were analyzed by immunoblotting with indicated antibodies. Related to Figure 2c. (**d**) The protein amounts in WT and *Rip3* KO L929 cells knocked down with shPpm1b or control shRNAs were analyzed by immunoblotting with indicated antibodies. Related to Figure 2f. (**e**) The protein amounts in WT and *Rip3* KO mouse peritoneal macrophage cells knocked down with shPpm1b or control shRNAs were analyzed by immunoblotting with indicated antibodies. Related to Figure 2g. For Figure a,c,d,e, data shown were representative of two or more independent experiments. The asterisk (*) denotes a nonspecific band. Statistics source data for this figure can be found in Supplementary Table 2. Uncropped images of blots are shown in Supplementary Figure 7.



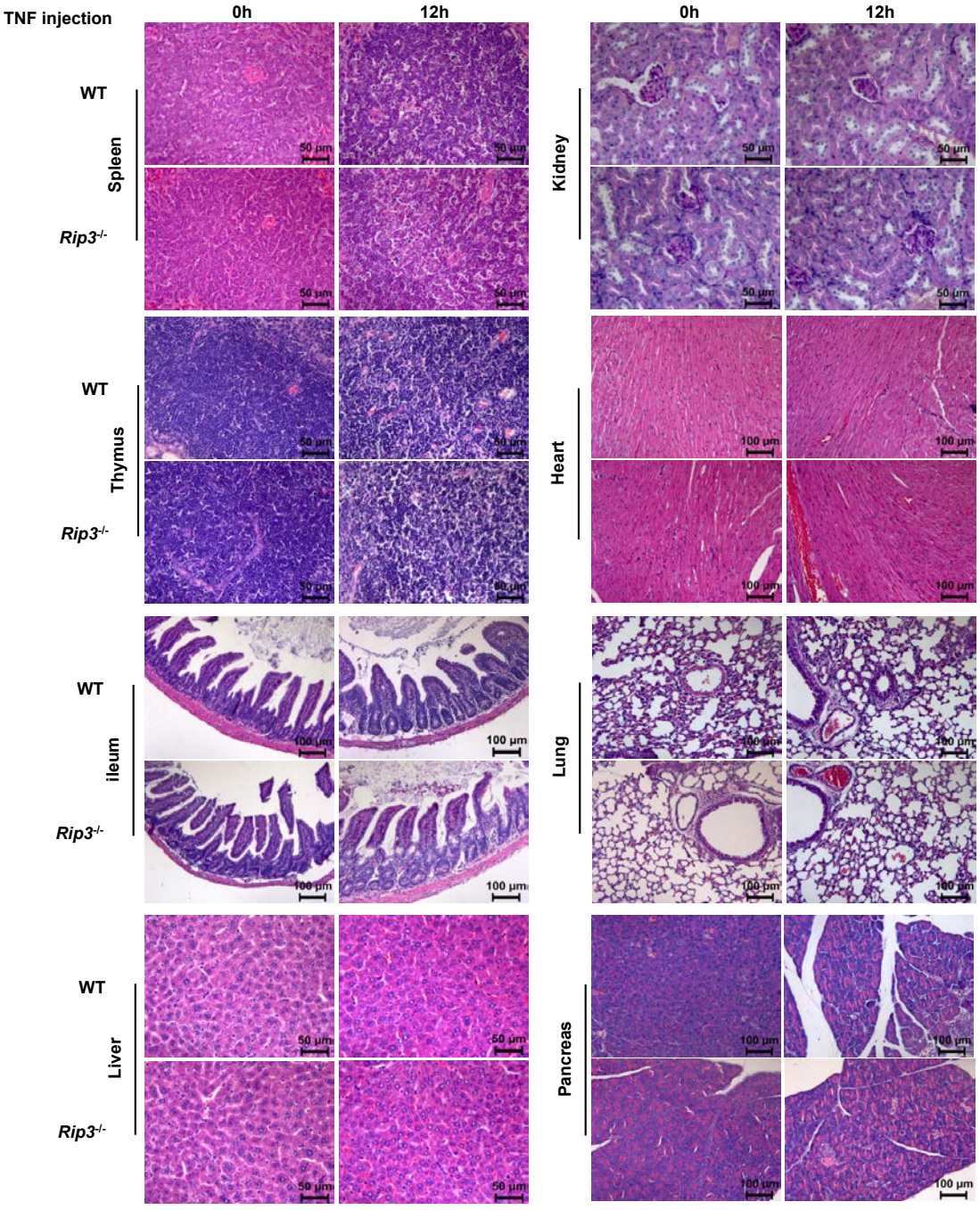
Supplementary Figure 3 Related to Figure 3. (**a**) Ppm1b was knocked down in *Rip3* KO L929 cells reconstituted with Rip3-WT, Rip3 phosphorylation deficient mutant (Rip3-2A) mutant and Rip3 RHIM domain mutant ($Rip3^{RHIM}$). The cells were lysed and subjected to immunoblotting with indicated antibodies. Related to Figure 3b. (**b**) Ppm1b was knocked down in WT and *Rip1* KO L929 cells. The cells were lysed and subjected to immunoblotting with indicated antibodies. The vertical line represents a splice mark. The spliced images were from the same blot. Related to Figure 3c. (**c**) WT Rip3 and $Rip3^{RHIM}$ were introduced into *Rip1* KO and WT L929 cells by lentiviral vectors. The cells were lysed and subjected to immunoblotting with indicated antibodies. Related to Figure 3d. (**d**) Ppm1b was knocked down in WT and *Mlkl* KO L929 cells. The cells were lysed and subjected to immunoblotting with indicated antibodies. Related to Figure 3h. (**e**) *TNFR1* KO and WT L929 cells were infected with lentivirus encoding shPpm1b or control. Left, spontaneous cell death was analyzed 48 hours later. n = 3000 cells pooled from 3 independent experiments. Right, *TNFR1* KO and WT L929 cells were treated with or without TNF for 12 hours. Cell death was analyzed. n = 3 independent experiments. Results shown were mean ± s.e.m.; $^{##}P<0.01$; $^{###}P<0.001$. For Figure a-d, data shown were representative of two or more independent experiments. The asterisk (*) denotes a nonspecific band. Statistics source data for this figure can be found in Supplementary Table 2. Uncropped images of blots are shown in Supplementary Figure 7.



Supplementary Figure 4 Ppm1b targets TNF-induced necroptosis but not apoptosis. (**a-b**) L929 cells were infected with lentivirus encoding shPpm1b or control, or not infected (Mock). 48 hours later, (a) the cells were treated with TNF (10 ng/ml) for indicated time periods and cell death was analyzed by flow cytometer; (b) the cells were treated with different doses of TNF as indicated for 6 hours and cell death was analyzed. n = 3 independent experiments. (**c**) Ppm1b does not affect TNF-induced apoptosis in NIH3T3-A cells. Ppm1b was knocked down in NIH3T3-A cells. Left, 48 hours later, the cells were treated with or without TNF (100 ng/ml) for 24 hours and cell death was analyzed. n = 3 independent experiments. Right, the cell lysates were analyzed by immunoblotting with indicated antibodies. (**d**) Both Ppm1b-L and S isoforms restrict TNF-induced necroptosis in L929. Ppm1b-S, Ppm1b-L or both isoforms were knocked down with isoform specific shRNAs in L929 cells. 48 hours later, the cells were treated with or without TNF (10 ng/ml) for 6 hours and cell death was measured. n = 3 independent experiments. (**e**) *Ppm1b* KO L929 cells were infected with different doses of lentivirus encoding Ppm1b-L and Ppm1b-S as indicated, or not infected (Mock infection). Then the cells were treated with TNF (10 ng/ml) for 6 hours and cell death was analyzed. n = 3 independent experiments. (**f**) Ppm1b does not affect the TNF-induced p65 and p38 phosphorylation. Ppm1b was knocked down with shRNA in L929 cells. 48 hours later, the cells were treated with TNF (10 ng/ml) for indicated time. The cell lysates were analyzed by immunoblotting with indicated antibodies. (**g**) The Ppm1b-β-geo fusion mRNA sequences in *Ppm1b*$^{d/d}$ mice were determined by 3' RACE. For Figure a-e, results shown were mean ± s.e.m.; $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$; N.S.: no significant difference. For Figure f, data shown were representative of two independent experiments. The asterisk (*) denotes a nonspecific band. Statistics source data for this figure can be found in Supplementary Table 2. Uncropped images of blots are shown in Supplementary Figure 7.



Supplementary Figure 5 The regulation of necroptosis by Ppm1b is independent of NF-κB pathway. (**a**) HeLa cells were infected with lentivirus encoding sh-hPpm1b or control. 48 hours later, the cells were treated with hTNF (30 ng/ml) for indicated time and subjected to immunoblotting with indicated antibodies. (**b**) HT29 cells were analyzed as in (a). (**c**) L929 cells were infected with lentivirus encoding shPpm1b or control, or not infected (Mock). 48 hours later, the cells were treated with TNF for indicated time and the IL-6 level of the cell culture supernatant was analyzed by ELISA. n = 3 independent experiments. (**d**) Left, L929 cells were infected with lentivirus encoding shPpm1b or control, or not infected (Mock). 5 hours after infection, the cells were treated with DMSO, TPCA-1 (1μM) or IMD 0354 (5μM). 48 hours later, spontaneous cell death was analyzed. Middle, *IKKβ* KO and WT L929 cells were infected with lentivirus encoding shPpm1b or control, or not infected. 48 hours later, spontaneous cell death was analyzed. Right, the *IKKβ* protein amount in *IKKβ* KO and WT L929 cells was determined by immunoblotting. n = 3000 cells pooled from 3 independent experiments. (**e**) Left, the cells were treated as in (d, left), except that the cells were treated with TNF for 5 hours and the TNF-induced cell death rather than spontaneous cell death was analyzed 48 hours after infection. Right, the cells were treated as in (d, middle), except that the cells were treated with TNF for 5 hours and TNF-induced cell death rather than spontaneous cell death was analyzed 48 hours after infection. n = 3 independent experiments. (**f**) L929 cells were pretreated with DMSO, TPCA-1 or IMD 0354 for 2 hours followed by treatment with TNF for indicated time. The IL-6 level of the cell culture supernatant was analyzed by ELISA. n = 3 independent experiments. (**g**) L929 cells were infected with lentivirus encoding shPpm1b or control, or not infected. 48 hours later, the cells were pretreated with DMSO or 5z-7 (1 μm) for 2 hours followed by treatment with TNF for 5 hours. Then the cell death was analyzed. n = 3 independent experiments. (**h**) HeLa cells stably expressing human Rip3 (HeLa-hRip3) were analyzed as in (a). (**i**) HeLa-hRip3 cells were infected with lentivirus encoding sh-hPpm1b or control, or not infected. Spontaneous cell death and hTNF (30 ng/ml) +Smac mimetic (100 nM) +zVAD (20 μM) (TSZ)-induced cell death were analyzed as in (d, left) and (e, left). n = 3 independent experiments. (**j**) Littermates of *Ppm1b*$^{d/d}$ and WT mice were injected with TNF (15 μg) via the tail vein. Then the serum IL-6 level was analyzed by ELISA at different time points as indicated. n=4 mice for each group in single experiment where two independent experiments were performed to assess reproducibility. ND: not detectable. For Figure c-g,i,j, results shown were mean ± s.e.m.; $^{\#}$P<0.05; $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001;. For Figure a,b,h, data shown were representative of two or more independent experiments. Statistics source data for this figure can be found in Supplementary Table 2. Uncropped images of blots are shown in Supplementary Figure 7.



Supplementary Figure 6 TNF induces tissue damage in different organs of WT and *Rip3*$^{-/-}$ mice. Littermates of WT and *Rip3*$^{-/-}$ mice were injected with TNF (15 μg) via the tail vein for indicated time. The sections of kidney were analyzed by PAS staining while those of the other organs were analyzed by H&E staining. The representative images were shown (n=5 mice of each genotype at 12 hour time point; n=3 mice of each genotype at 0 hour time point.). Scale bar: 50 or 100 μm as indicated.

Supplementary Figure 7 Uncropped images of blots in Figure 1-7 and Supplementary Figure 1-6.

Supplementary Table Legends

Supplementary Table 1 The mass spectrometric data of Rip3 immunocomplex. Rip3 KO L929 cells were reconstituted with Flag-Rip3 to generate Rip3-KO-Flag-Rip3 L929 cells. Upon TNF treatment for 5 hours, these cells were lysed and subjected to immunoprecipitation with anti-Flag antibody. Then the immunocomplex was analyzed by mass spectrometry and results were listed below. See "Methods" for detail. Protein score: A measure of the protein confidence for a detected protein, calculated from the peptide confidence for peptides. The higher the value is, the more confidently the protein exists in the sample. %Cov (coverage): The percentage of matching amino acids from identified peptides having confidence greater than 0, divided by the total number of amino acids in the sequence. %Cov(95)(coverage): The percentage of matching amino acids from identified peptides having confidence greater than 95%, divided by the total number of amino acids in the sequence. Peptides(95%): The number of the unique, high confident (>95%) peptides in the detected protein.

Supplementary Table 2 The statistics source data for Figures and Supplementary Figures.