

## **RIPK1 dephosphorylation and kinase activation by PPP1R3G/PP1 $\gamma$ promotes apoptosis and necroptosis**

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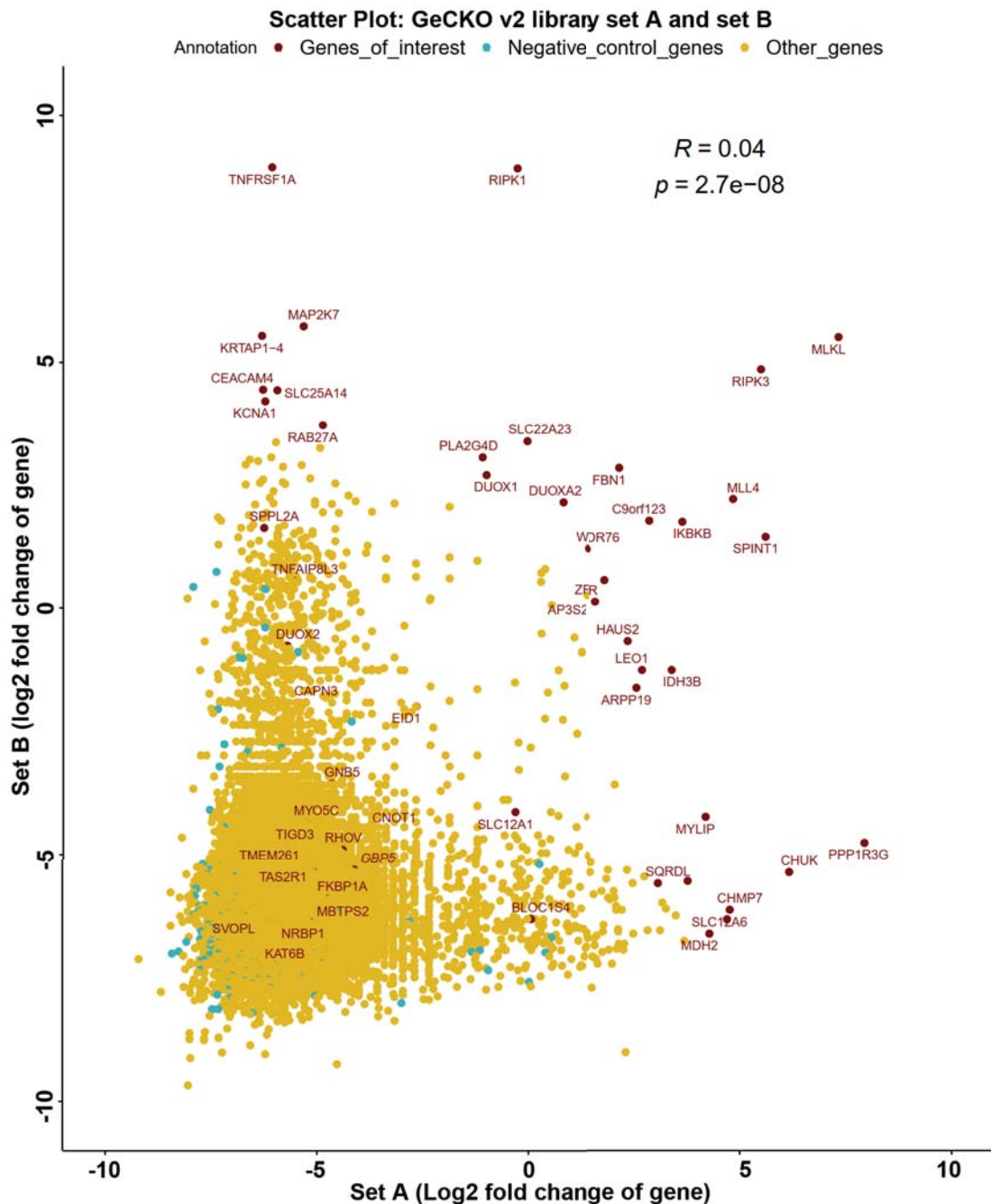
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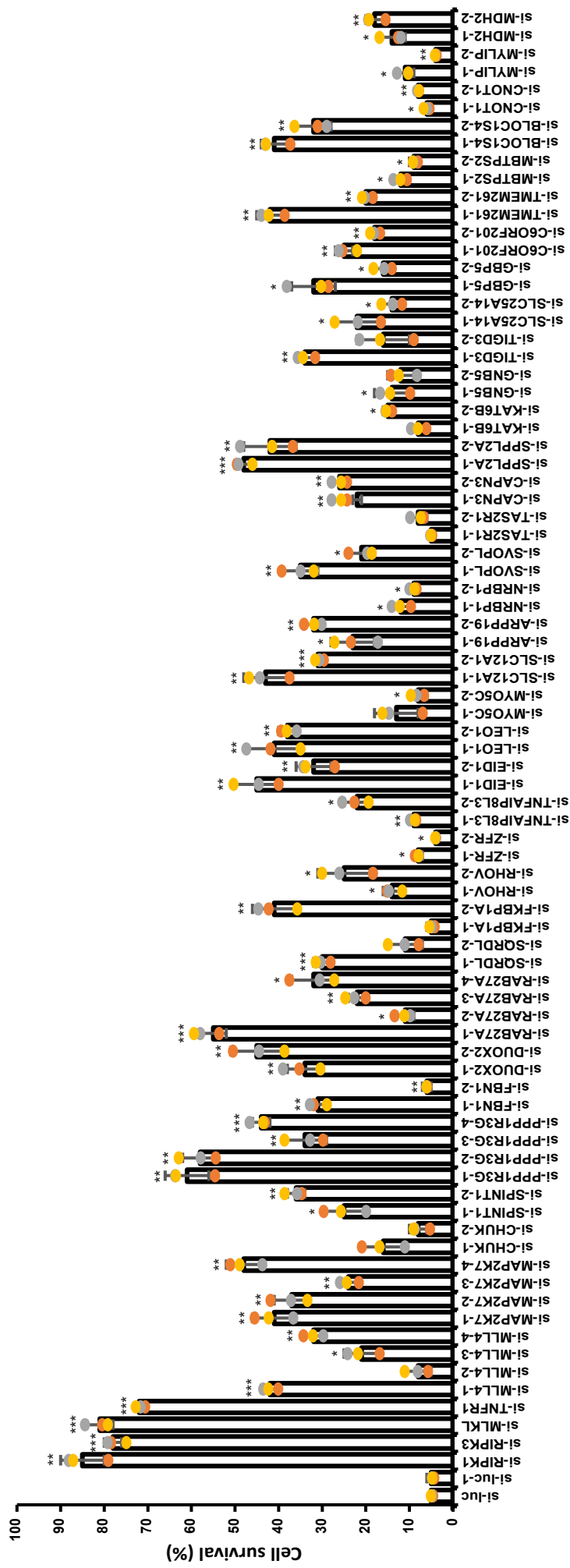
<sup>9</sup>These authors contribute equally

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## Supplementary figure 1



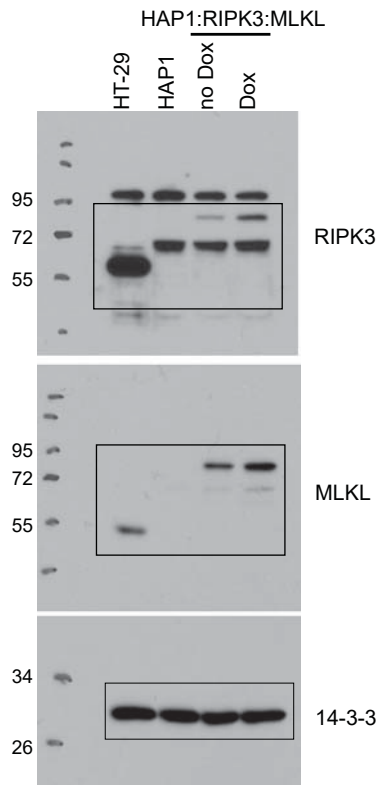
Supplementary Figure 1. Scatter plot for GeCKO v2 library set A and B. The GeCKO v2 library contains two sets of sgRNA for each gene, including 3 sgRNAs in library set A and 3 sgRNAs in library set B. Fold change between control and T/S/Z sample was calculated for each gene in set A and set B using MAGeCK and then plotted as a scatter plot. Spearman correlation coefficient and significance of correlation is shown in the plot. We first randomly subsetting the negative control guides into dummy genes. We then used MAGeCK test command with input options “control-sgrna option” and “norm-method control” ([https://sourceforge.net/p/mageck/wiki/QA/#what-does-the-control-sgrna-control\\_sgrna-option-do-how-to-use-this-option](https://sourceforge.net/p/mageck/wiki/QA/#what-does-the-control-sgrna-control_sgrna-option-do-how-to-use-this-option)). MAGeCK uses the list of negative control guides to generate the null distribution of RRA score during p-value calculation. As per the recommendation from authors of MAGeCK we used 1000 negative control guides for p value estimation. P-value significance of  $2.7e-08$  was found between set A and set B.



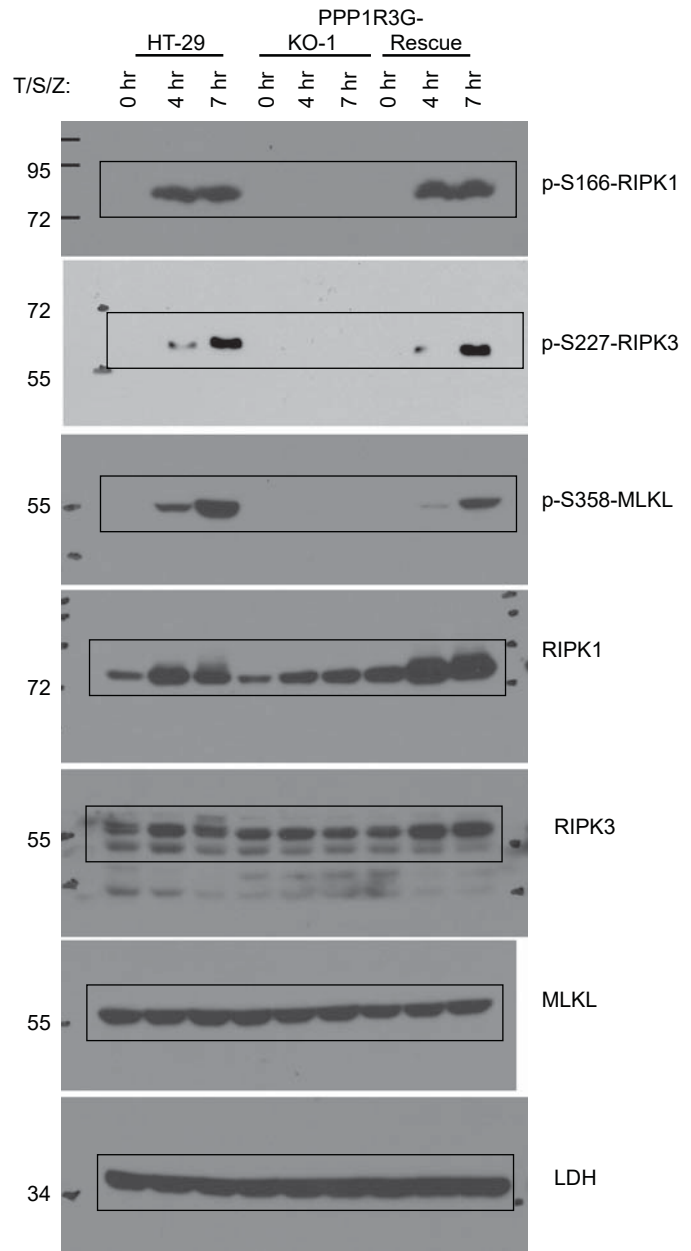
Supplementary Figure 2. Secondary siRNA screen. Small interfering RNAs (siRNA) were transfected into HAP1:RIPK3:MLKL cells for 48 hours followed by DMSO or T/S/Z treatment for 16 hours. Cell survival was measured with CellTiter-Glo assay. Viable cells expressed as a percentage of DMSO-treated cells. Results are represented as mean  $\pm$  SD of n=3 biological independent samples. Two-tailed Student's t-test, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

Supplementary figure 3. Source data immunoblots.

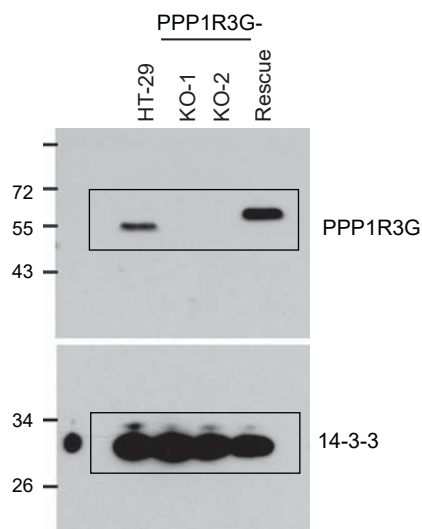
**Fig. 1a**



**Fig. 2j**

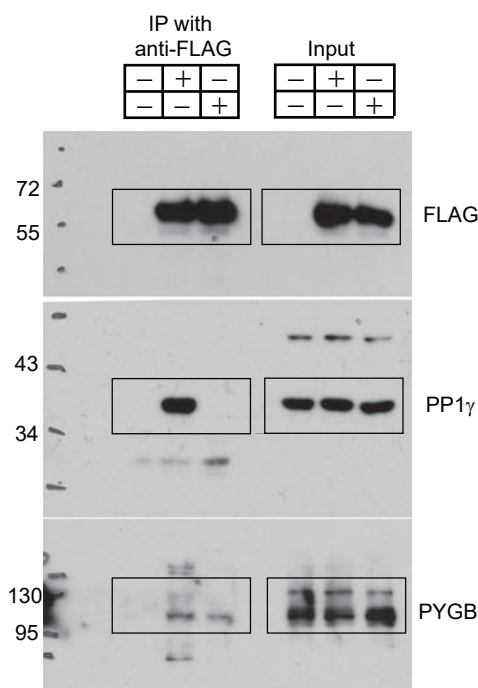


**Fig. 2c**

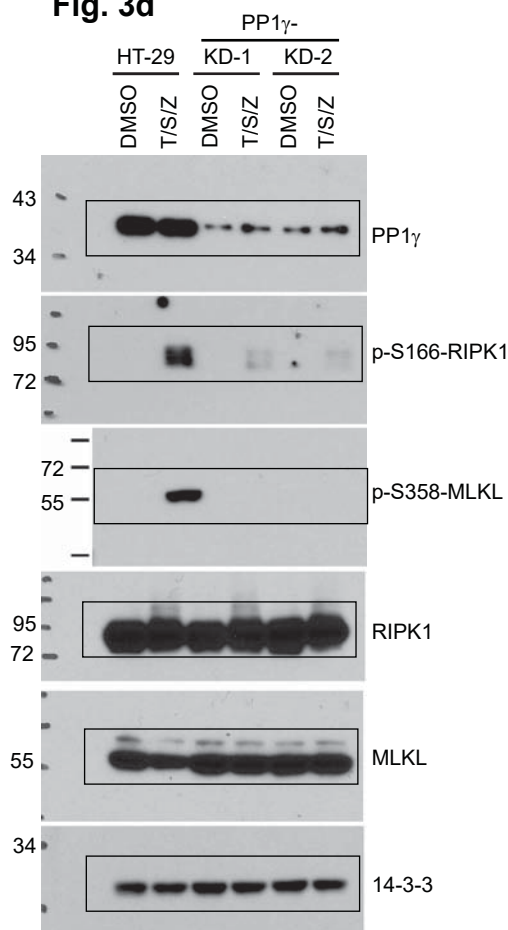


Supplementary figure 3. Source data immunoblots (continued).

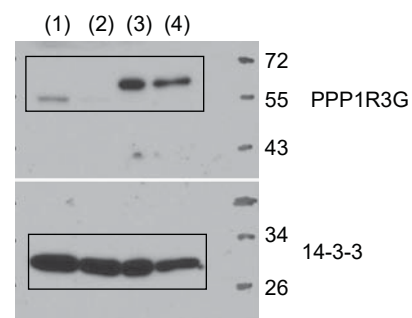
**Fig. 3b**



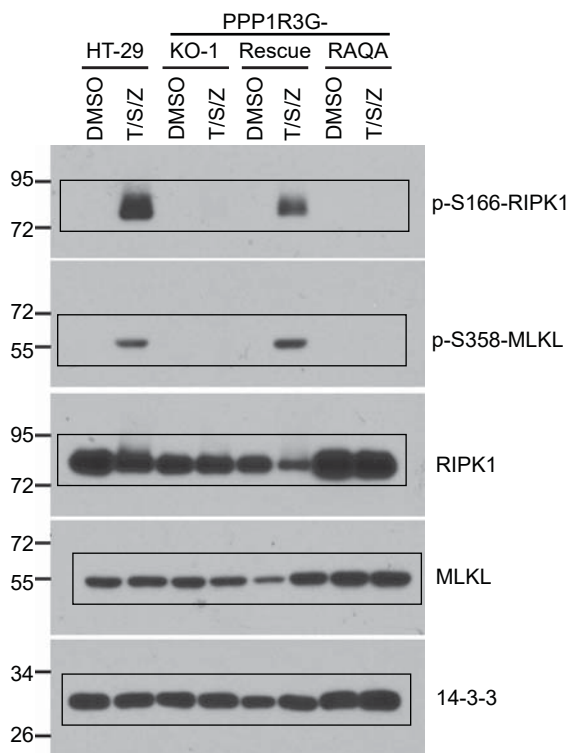
**Fig. 3d**



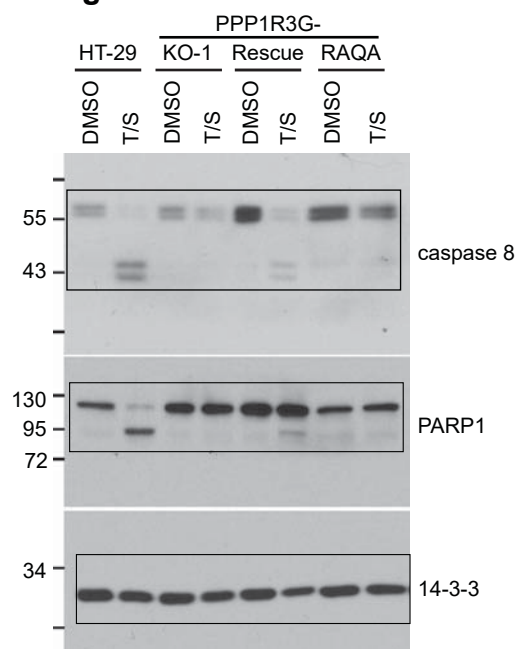
**Fig. 3e**



**Fig. 3f**

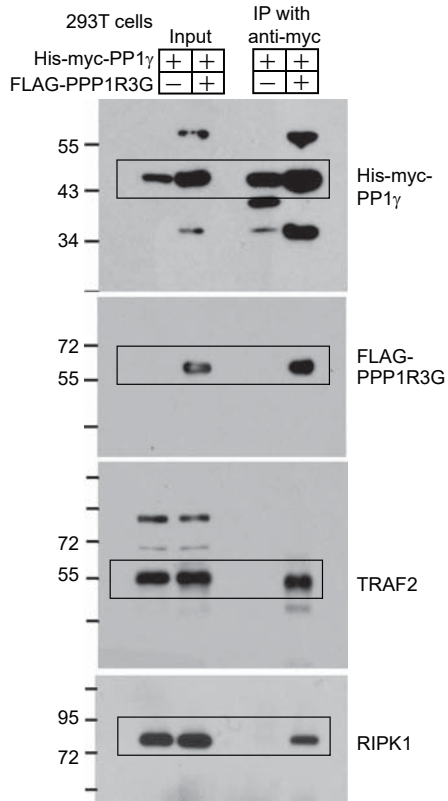


**Fig. 3h**

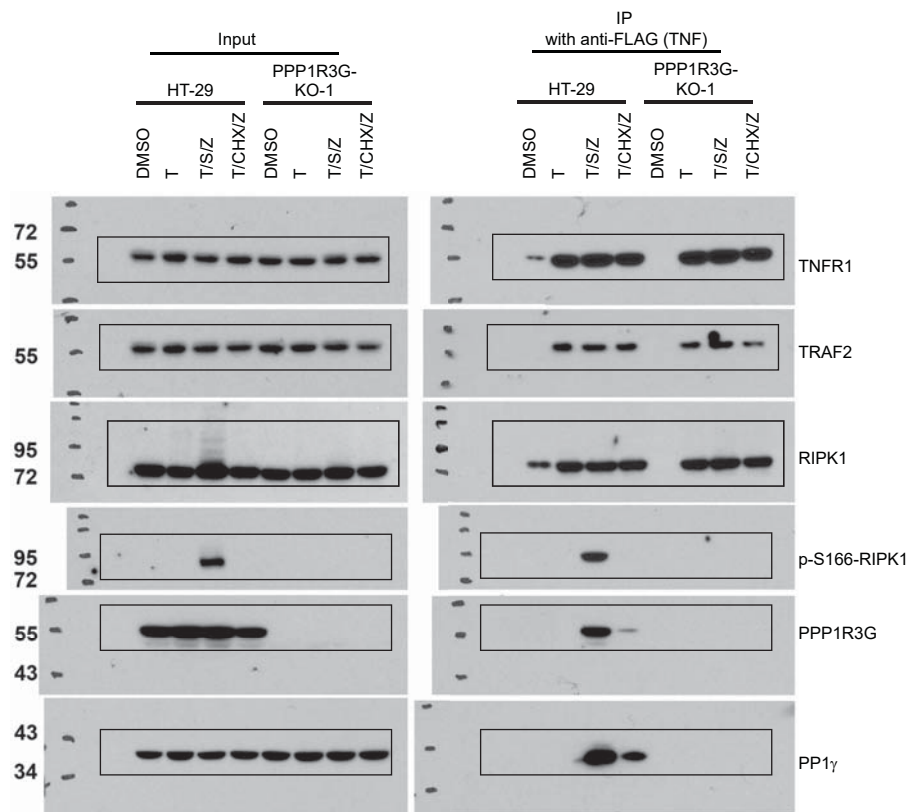


Supplementary figure 3. Source data immunoblots

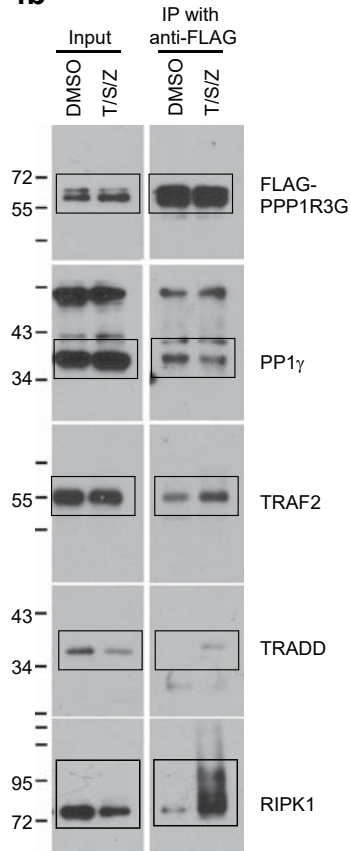
(continued). **Fig 4a**



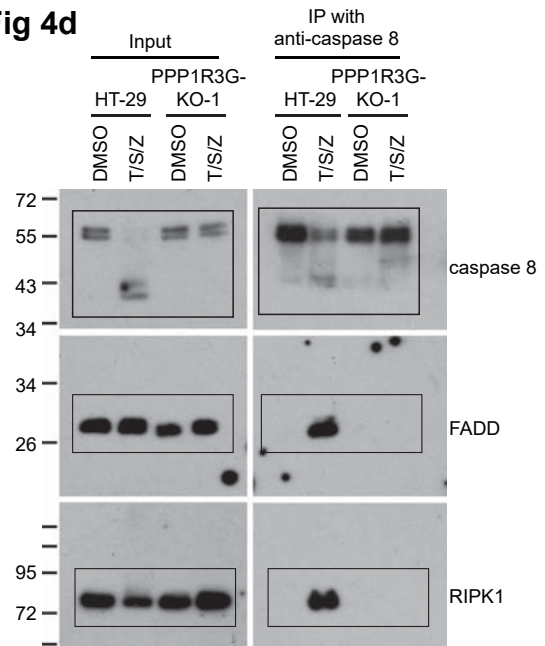
**Fig 4c**



**Fig 4b**

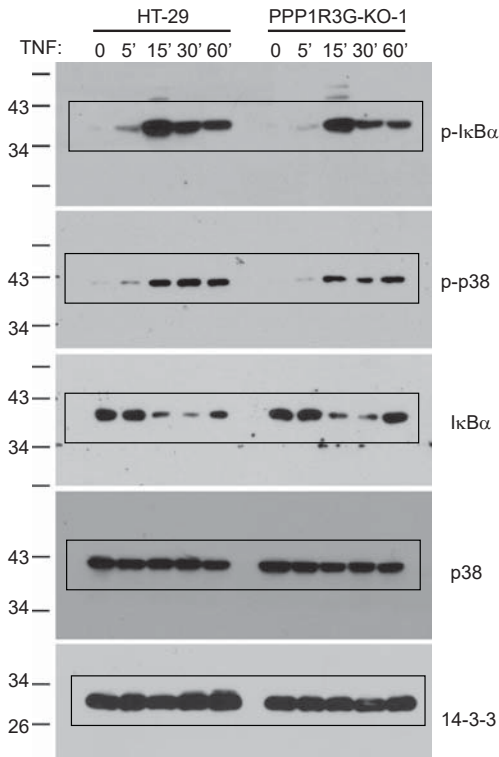


**Fig 4d**

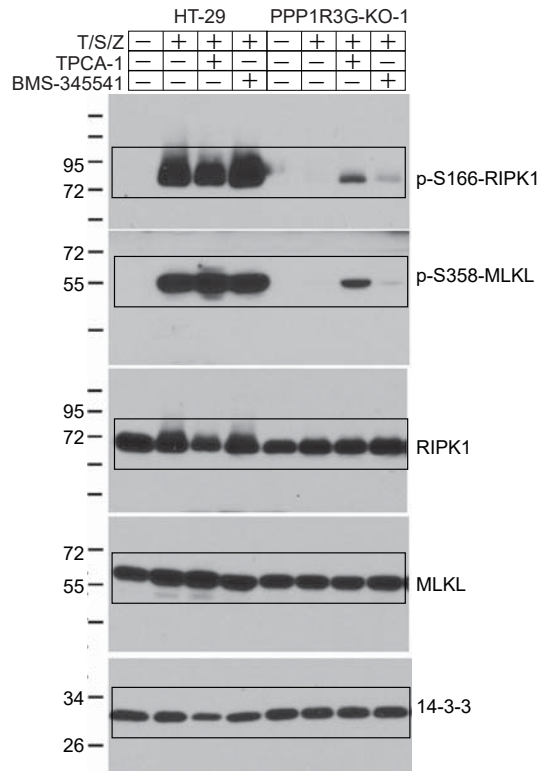


Supplementary figure 3. Source data immunoblots (continued).

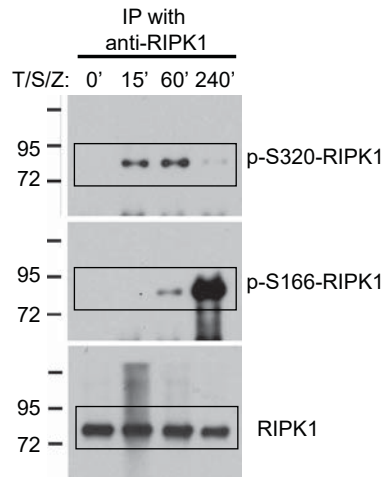
**Fig 5a**



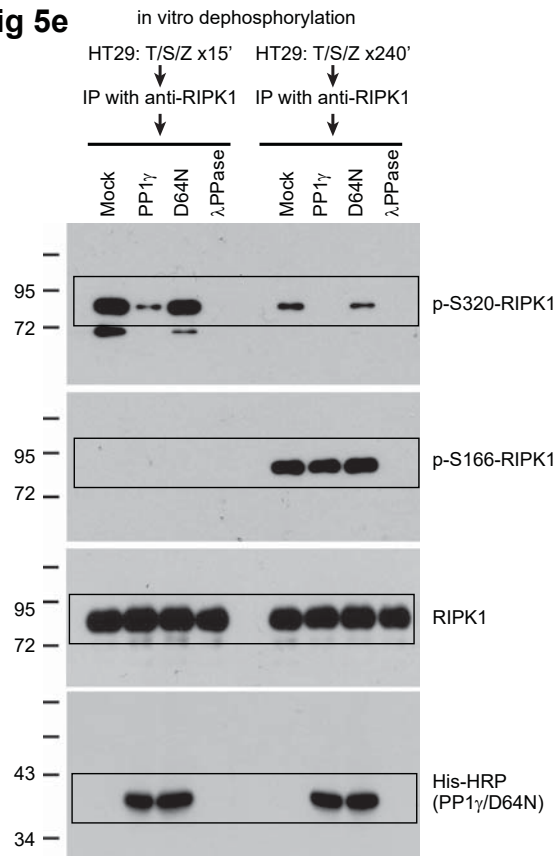
**Fig 5c**



**Fig 5d**

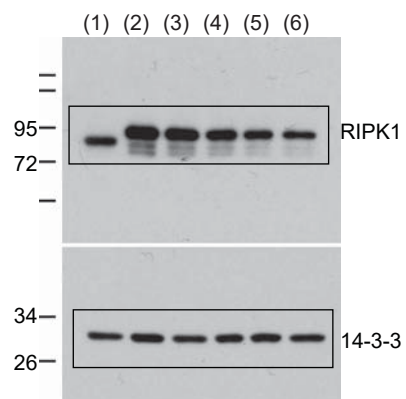


**Fig 5e**

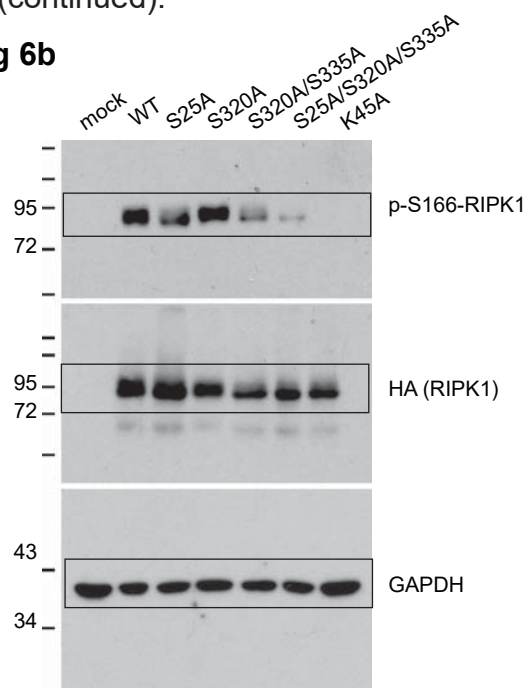


Supplementary figure 3. Source data immunoblots (continued).

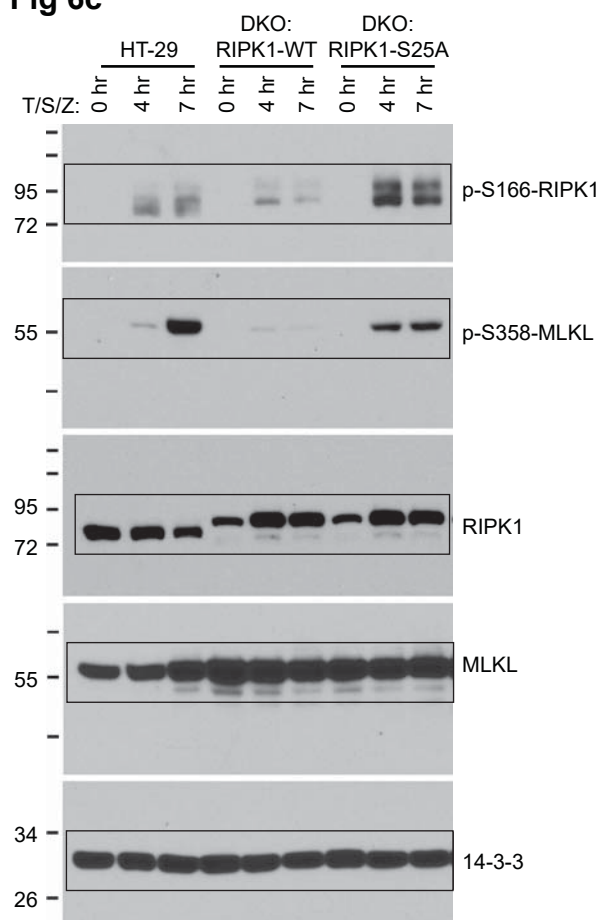
**Fig 6a**



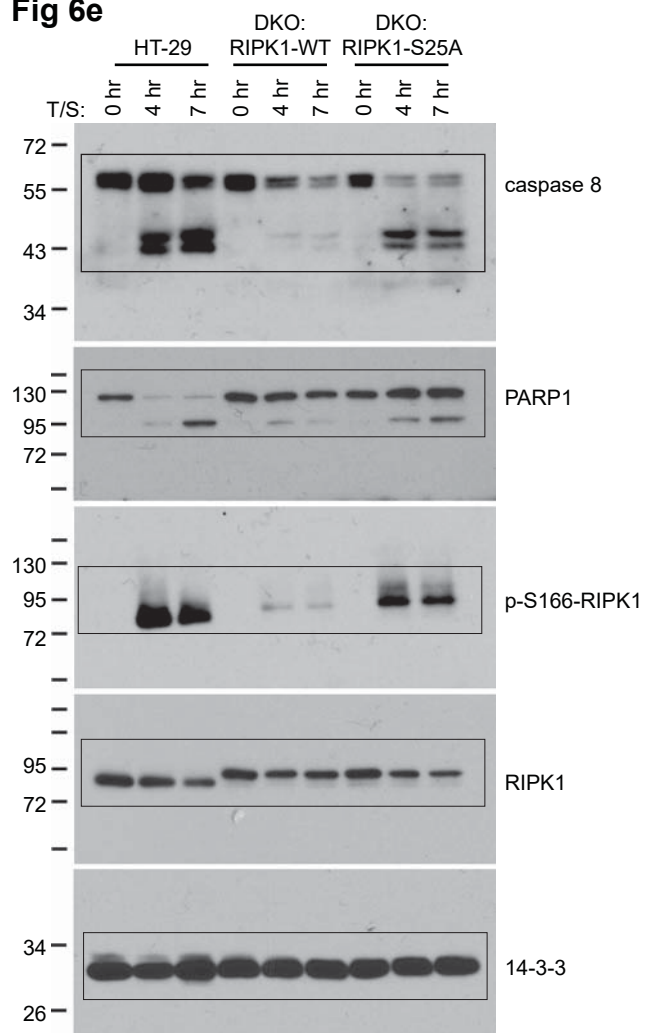
**Fig 6b**



**Fig 6c**



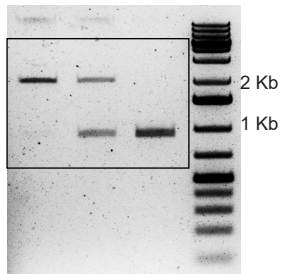
**Fig 6e**



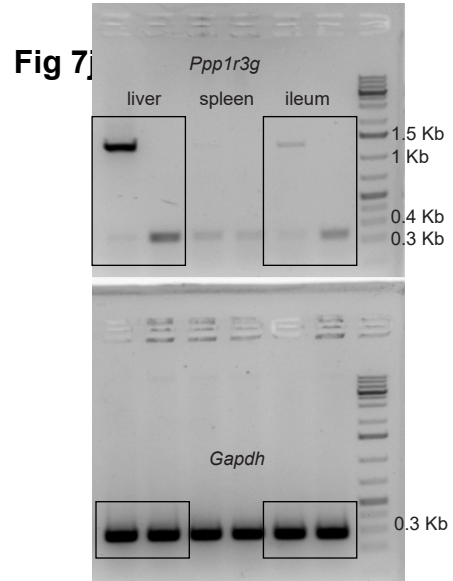


Supplementary figure 3. Source data immunoblots (continued).

**Fig 7b**



**Fig 7c**



**Fig 7e**

