RIPK1, RIPK3 and MLKL are activated by sublytic complement and participate in complement-dependent cytotoxicity

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Supplementary figures

Figure S1

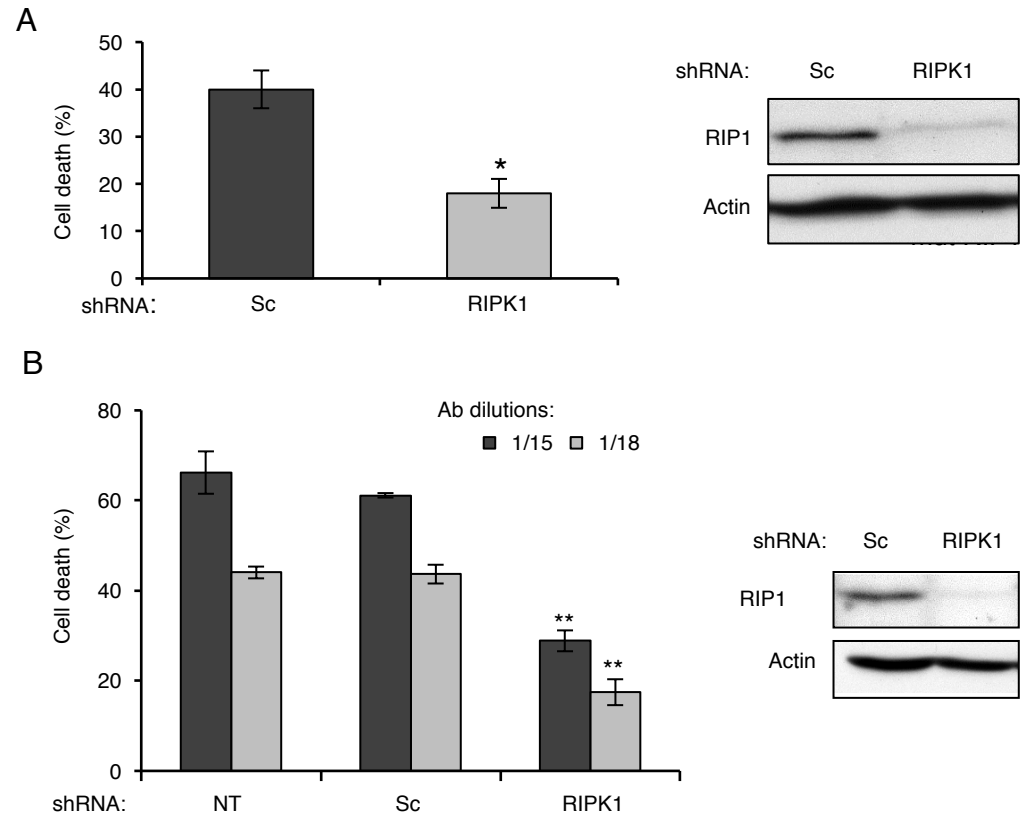

Figure S1. Silencing of RIPK1 by shRNA reduces CDC.

(A) HEK-293T cells transfected for 48 h with RIPK1-shRNA or a scrambled (Sc) shRNA or **(B)** K562 cells stably transfected with RIPK1 shRNA or scrambled shRNA were subjected to antibody and complement (NHS, 50%) treatment for 60 min at 37°C and cell death was measured by PI inclusion. Results of 3 independent experiments are expressed as mean percent cell death ± SD. **P*< 0.05, ***P*< 0.001 relative to cells expressing scrambled shRNA. Knockdown efficiency of RIPK1 was examined by Western blotting of cell lysates with anti-RIPK1 or anti-actin antibodies (right inserts). NT, non-treated.

Figure S2

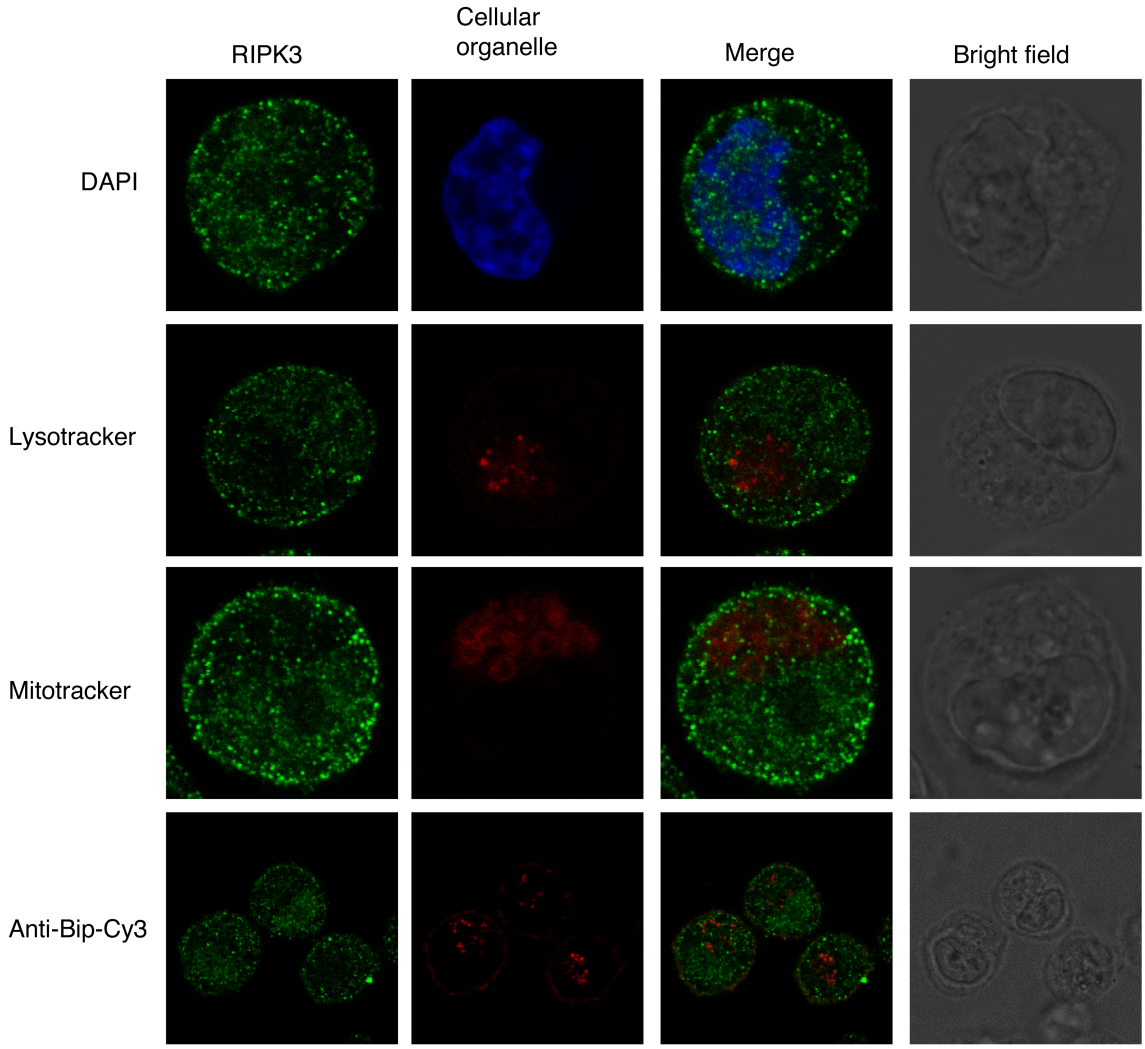

Figure S2. RIPK3 aggregates are not located within cellular organelles.

K562 Flag-RIPK3 transfected cells were treated for 10 min with complement at a sublytic dose, followed by fixation with 2% paraformaldehyde and permeabilization with 0.1% saponin. Flag-RIPK3 was immunostained with AF-488-labeled anti-Flag antibody. Nuclei, lysosomes and mitochondria were stained with DAPI, LysoTracker and MitoTracker, respectively. ER was stained with anti-GRP78 Bip antibody, followed by Cy3 labeled secondary antibody.