Figure S1

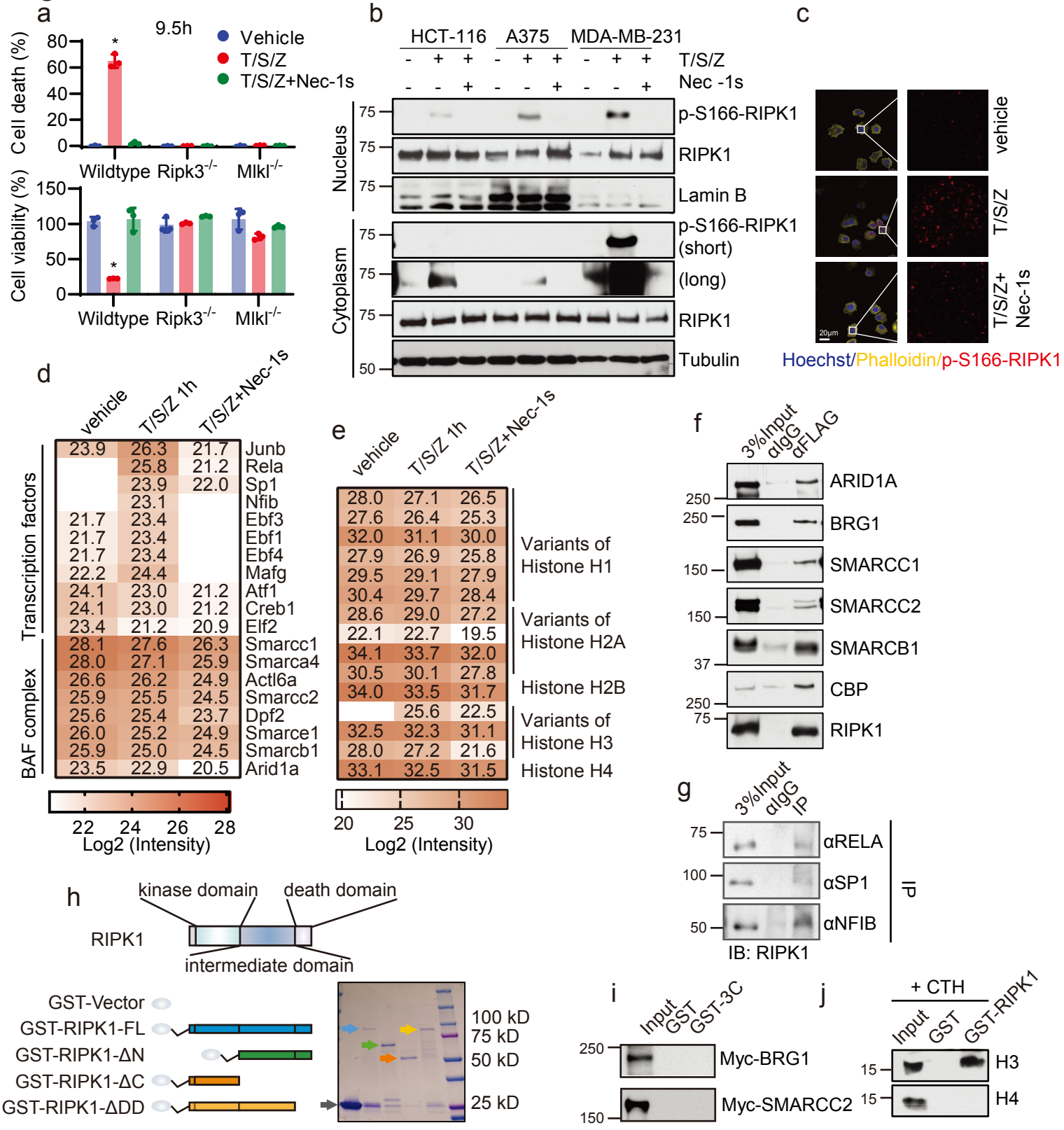

Fig. S1 Analyses of the nuclear RIPK1 interactome. a Wildtype, *Ripk3*$^{-/-}$, *Mlkl*$^{-/-}$ MEFs were treated with 50 μM SM164, 20 μM zVAD-fmk with or without Nec-1s for 0.5h and followed by 5 ng/ml mTNFα for 9.5h. Cell death was measured by SytoxGreen positivity. Cell viability was assessed by CellTiter-Glo assay. **b** HCT-116, A375, MDA-MB-231 cells were pre-incubated with 50 μM SM164, 20 μM zVAD-fmk, +/- 10 μM Nec-1s for 0.5h and then treated with 5 ng/ml mTNFα for 2h. The fractions of nuclear or cytoplasmic lysates were analyzed by IB. **c** Immunostaining of p-S166-RIPK1 in A375 cells treated with 50 μM SM164, 20 μM zVAD-fmk for 0.5h and then treated with 5 ng/ml mTNFα for 1h. Representative images with zoomed nuclear p-S166-RIPK1 are shown. Cell membrane was stained with Phalloidin (yellow). Nuclei were stained with Hoechst (blue). **d, e** Heatmap of quantitative mass spec-called intensity of selected RIPK1 binding proteins from MEFs treated with vehicle or 50 μM SM164, 20 μM zVAD-fmk, +/- 10 μM Nec-1s for 0.5h and then treated with 5 ng/ml mTNFα for 1h. Blank represents no detection for the protein indicated. See also Table S1. **f** *Ripk1*$^{-/-}$ MEFs were infected with FLAG-Ripk1 lentivirus. Whole cell lysates were immunoprecipitated with anti-FLAG M2 beads, eluted with FLAG peptides and followed by western blotting with antibodies against the indicated proteins. **g** FLAG-Ripk1 MEFs were treated with 50 μM SM164, 20 μM zVAD-fmk for 0.5h and followed by 5 ng/ml mTNFα for 1h. Whole cell lysates were immunoprecipitated with the antibody of the indicated endogenous antibodies of transcription factors followed by western blotting with antibodies against RIPK1. **h** Schematic diagrams of the indicated GST fused full length (FL) or truncations of human RIPK1. Purified GST, GST-RIPK1, GST-RIPK1-ΔN, GST-RIPK1-ΔC and GST-RIPK1ΔDD proteins from E. coli were resolved by SDS-PAGE and stained with Coomassie Blue. **i** The result of GST pull-down assays with Myc-BRG1, Myc-SMARCC2 synthesized by in vitro transcription/translation, and GST, GST-3C purified from *E. coli*. **j** *In vitro* pulldown of calf thymus histones (CTH) with purified GST fused human RIPK1 was determined by western blotting with antibodies against Histone H3 and H4.