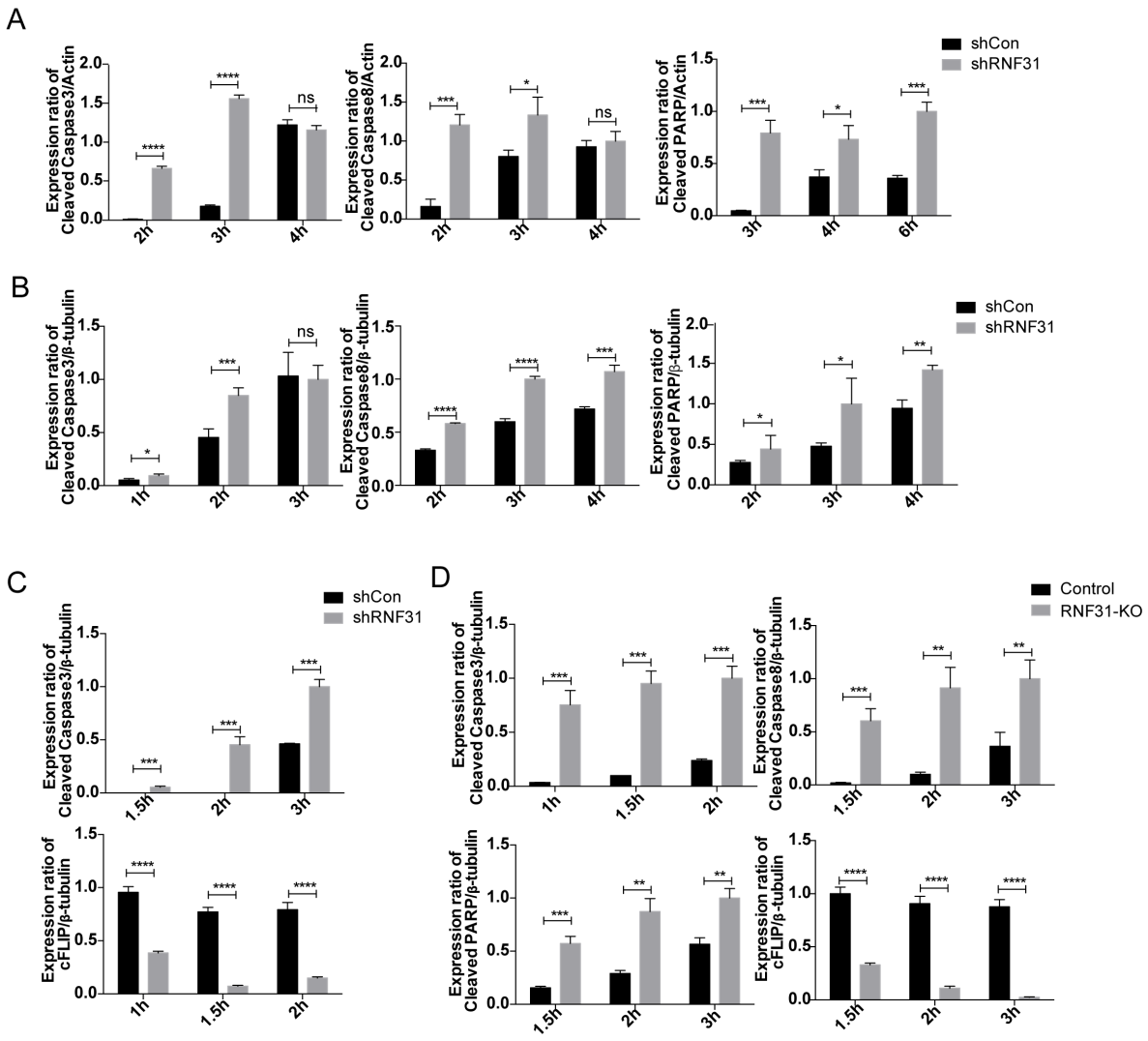
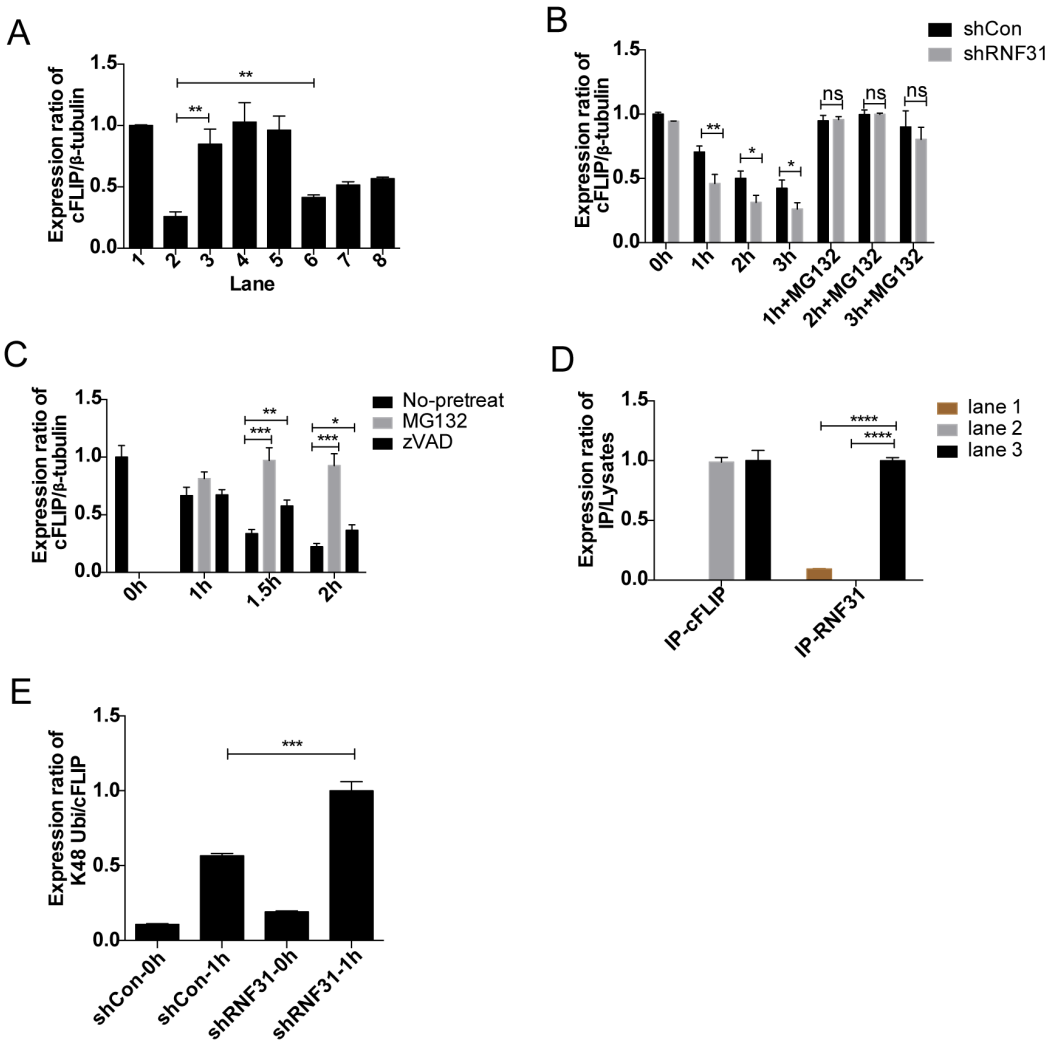
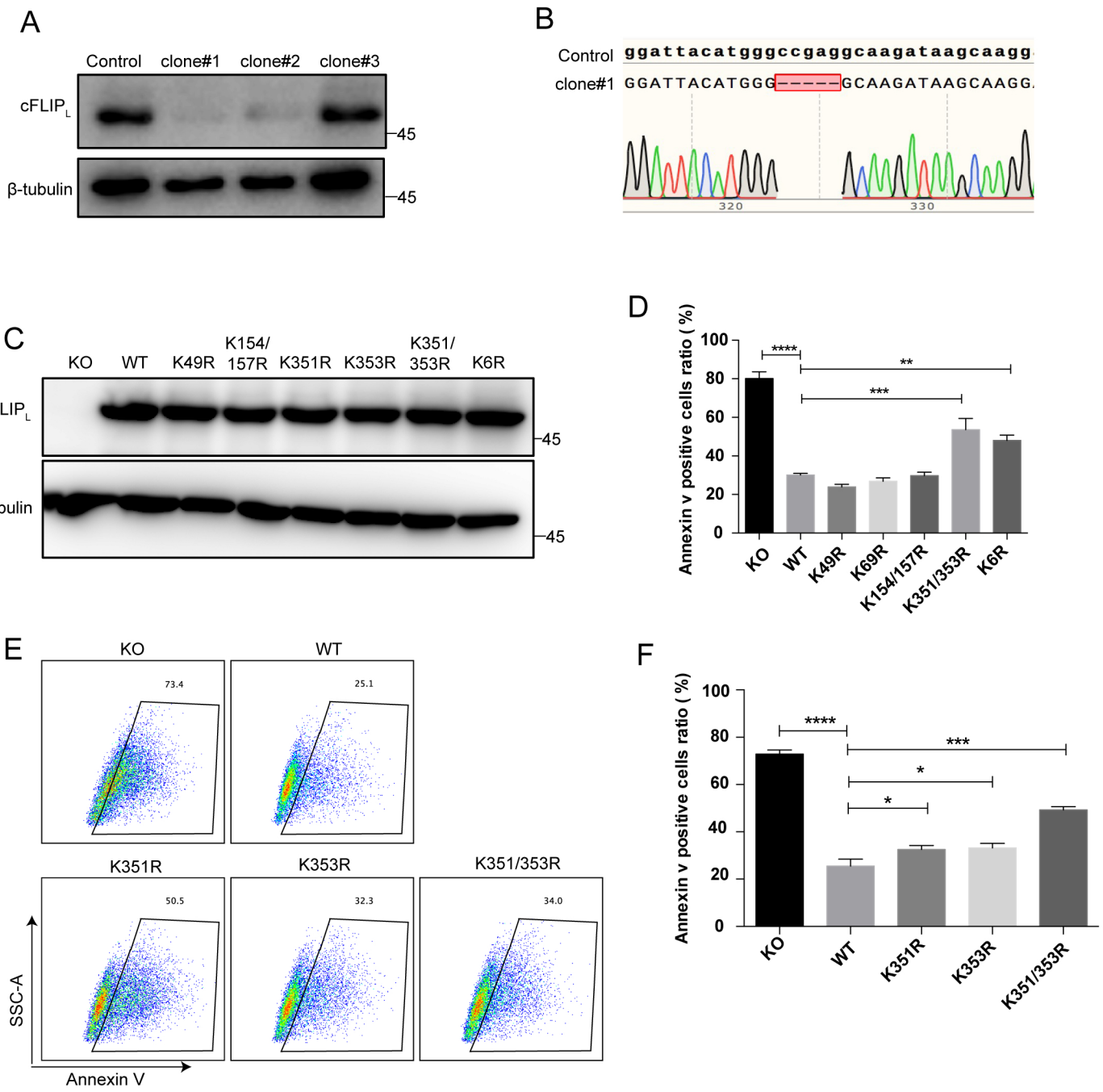
Tang et al, Supporting Information



Supplementary Figure S1. cFLIP degrades faster in RNF31-deficient cells. (A) Quantitation of cleaved Caspase3, cleaved Caspase8 and cleaved PARP in Figure 1A are shown. Values of cleaved Caspase3 and cleaved Caspase8 are relative to treated shRNF31 cells (4 hr), and values of cleaved PARP are relative to treated shRNF31 cells (6 hr). (B) Quantitation of cleaved Caspase3, cleaved Caspase8 and cleaved PARP in Figure 1B are shown. Values of cleaved Caspase3, cleaved Caspase8 and cleaved PARP are relative to treated shRNF31 cells (3 hr). (C) Quantitation of cleaved Caspase3 and cFLIP in Figure 1D are shown. Values of cleaved Caspase3 are relative to treated shRNF31 cells (3 hr), and values of cFLIP are relative to untreated shCon cells (0 hr). (D) Quantitation of cleaved Caspase3, cleaved caspase8, cleaved PARP and cFLIP in Figure 1E are shown. Values of cleaved Caspase3 are relative to treated RNF31-KO cells (2 hr), and values of cleaved Caspase8 and cleaved PARP are relative to treated RNF3-KO cells (3 hr). Values of cFLIP are relative to untreated Control cells (0 hr). In(A-D), n=3, data are mean± s.e.m. Statistical significance was determined using a two-tailed unpaired *t* test, ns P>0.05, P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



Supplementary Figure S2. RNF31 interacts with cFLIP to induce M1-linked ubiquitination on cFLIP. (A) Quantitation of cFLIP in Figure 2B are shown. Values of cFLIP are relative to untreated cells (0 hr, lane1). (B) Quantitation of cFLIP in Figure 2C are shown. Values of cFLIP are relative to untreated shCon cells (0 hr). (C) Quantitation of cFLIP in Figure 2D are shown. Values of cFLIP are relative to untreated Control cells (0 hr). (D) Quantitation of immunoprecipitated cFLIP and RNF31 in Figure 3A are shown. Values of IP/lysates ratio are relative to lane3. (E) Quantitation of K48 ubi of immunoprecipitated cFLIP in Figure 4C are shown. Values of K48 ubi/cFLIP ratio are relative to treated shRNF3 cells (1 hr). In(A-E), n=3, data are mean± s.e.m. Statistical significance was determined using a two-tailed unpaired *t* test, ns P>0.05, P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



Supplementary Figure S3. Lys351 and Lys353 are the main M1-linked ubiquitination sites of cFLIP. (A) Jurkat cells were subjected to deletion of the cFLIP gene using the CRISPR-Cas9 system, and infected cells with CRISPR-Cas9 virus were selected using puromycin (2μg/ml). After isolation of single cells via serial dilution, colonies that arose from single cells were subjected to WB analysis to monitor the expression of cFLIP. (B) Genome sequencing of cFLIP from Clone#1. (C) cFLIP KO Jurkat cells were rescued with WT and ubiquitination site mutants of cFLIP, and WB analysis of cFLIP expression in these cells. (D) The statistical results of apoptosis cells by FACS. After stimulated with TNFα/CHX (40 ng/ml and 10 μg/ml, respectively) for 4 hrs, apoptosis cells were analyzed by the annexin-V staining and FACS. (E) The representative pictures of FACS results. Rescued cFLIP Jurkat cells were stimulated with TNFα/CHX (40 ng/ml and 10 μg/ml, respectively) for 4 hrs, and then FACS analyzed the apoptosis cells. (F) The statistical results of (C). In (D-F), n=3, and statistical significance was determined using Two-tailed unpaired t-test (* P<0.05, **P<0.01, ***P<0.001, ****P < 0.0001).