Figure. S1

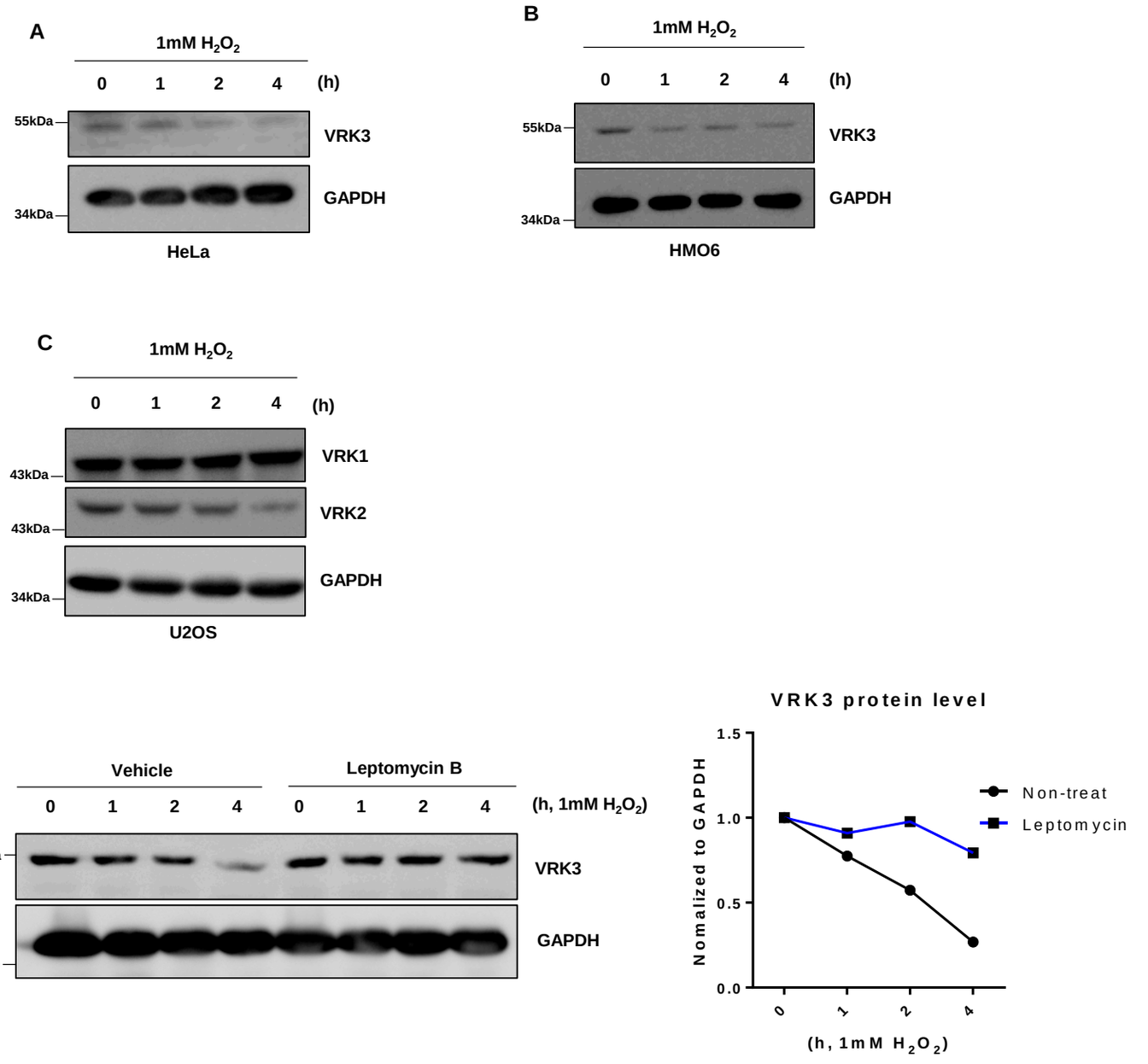

Figure S1. (A, B) The level of VRK3 were decreased under oxidative stress in HeLa (A) and HMO6 (B) cell lines. (C) The protein levels of VRK2, but not VRK1 were decreased in response to H_2O_2. U2OS cells were treated with 1mM H_2O_2 at indicated time points. Cell lysates were analyzed by immunoblotting. (D) Oxidative stress mediated VRK3 degradation was inhibited by leptomycin B treatment. U2OS cells were treated with 10ng/ml of leptomycin B. One hour after leptomycin B treatment, 1mM H_2O_2 was treated for indicated time. Cell lysates were analyzed by immunoblotting.

Figure. S2

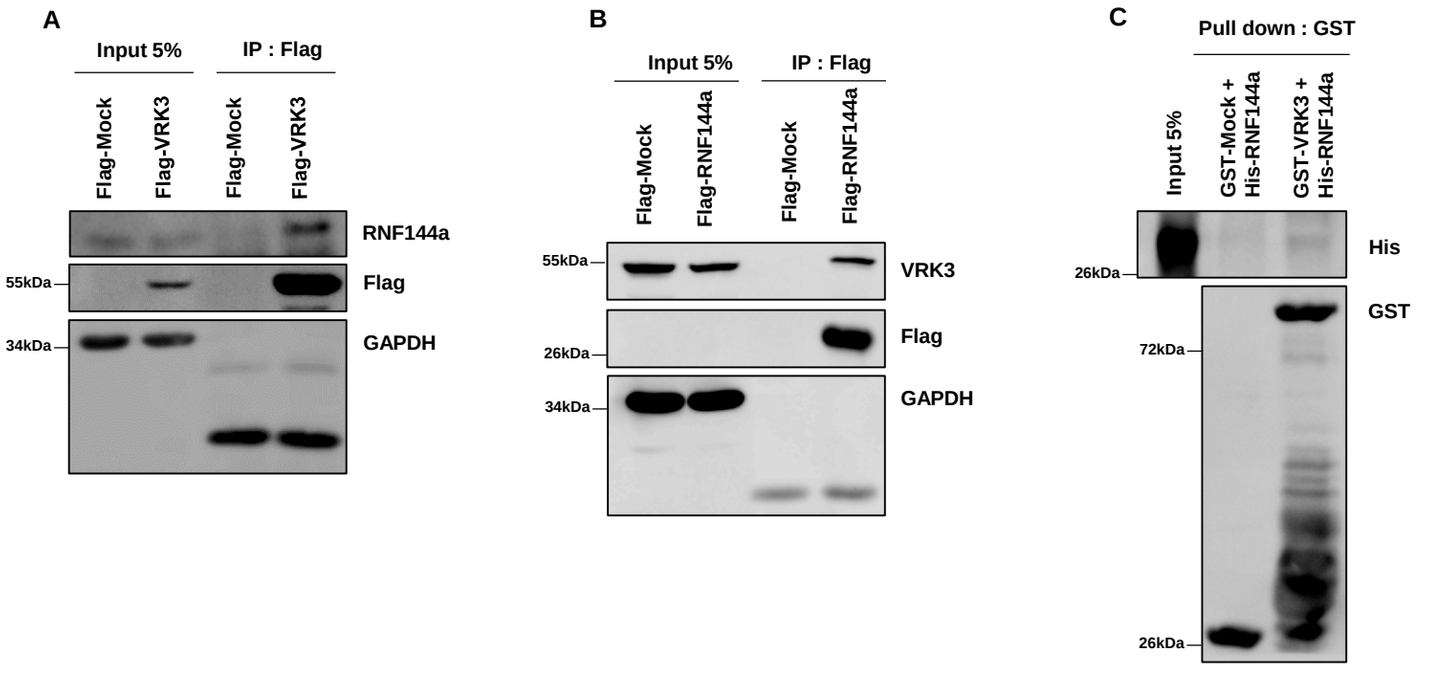

Figure S2. VRK3 interacts with RNF144a. (A) HEK293A cells were overexpressed with Flag-Mock or Flag-VRK3. Endogenous RNF144a were immunoprecipitated with anti-Flag antibody. (B) HEK293A cells were overexpressed with Flag-Mock or Flag-RNF144a. Endogenous VRK3 were immunoprecipitated with anti-Flag antibody. (C) VRK3 directly interacts with RNF144a. GST pull down assay were performed using recombinant proteins.

Figure. S3

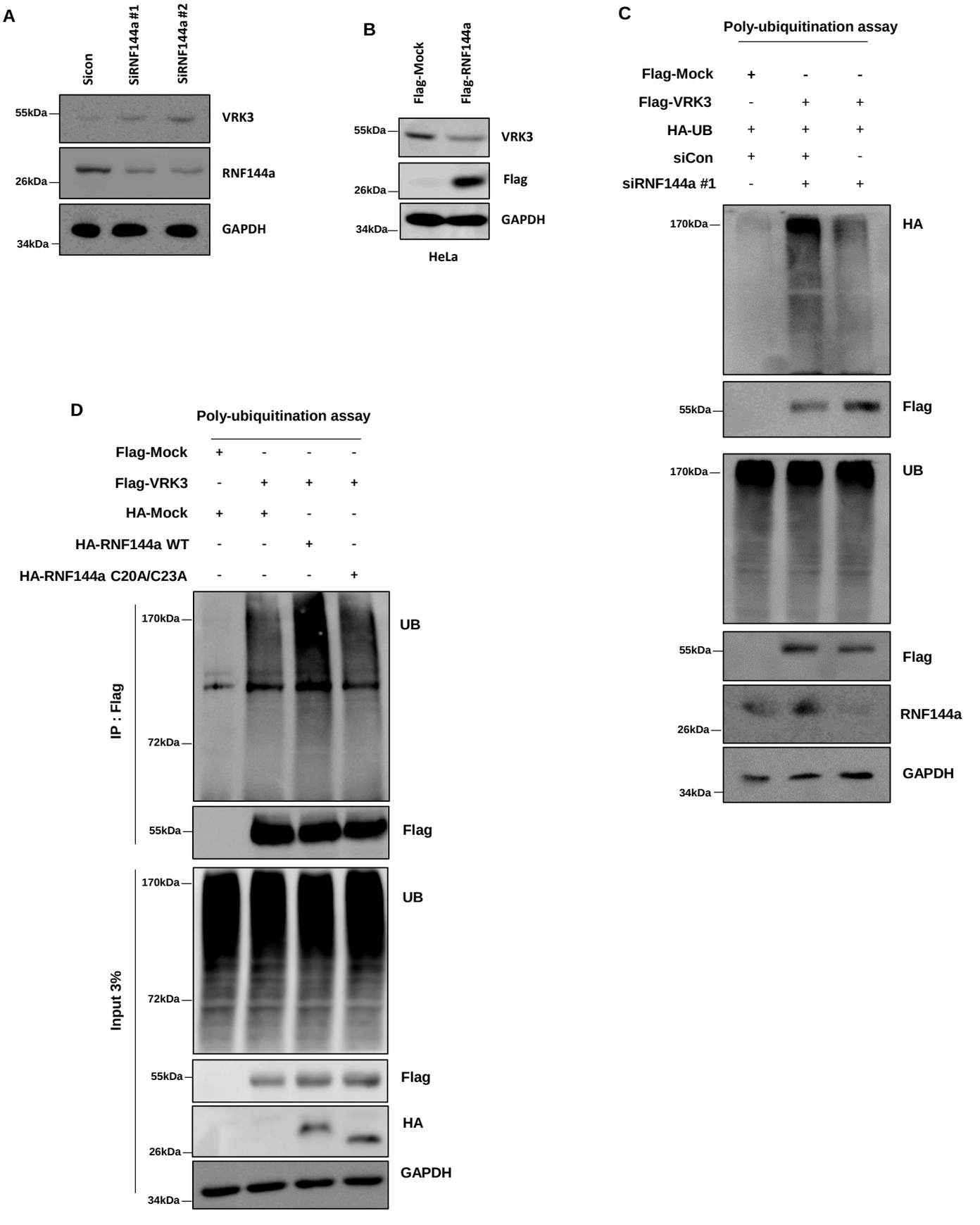

Figure. S3

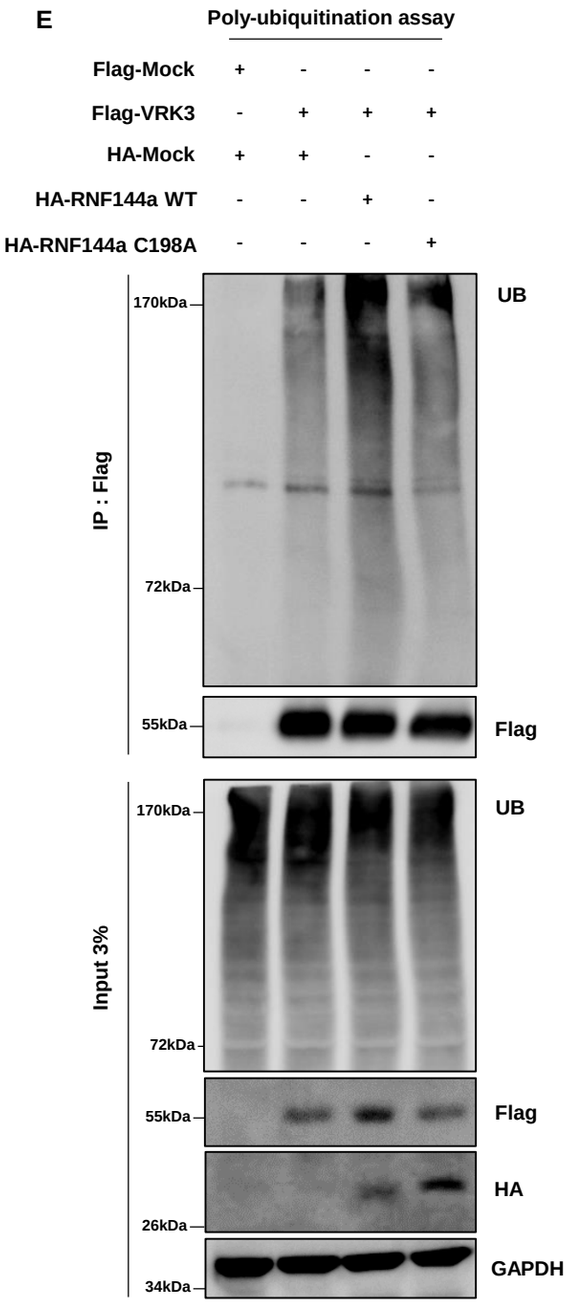

Figure S3. (A) Knockdown of RNF144a increases the level of VRK3. U2OS cells were transfected with siCon, siRNF144a#1 or siRNF144a#2. Cells were harvested 48 hours after transfection . Cell lysates were analyzed by immunoblotting. (B) RNF144a decreases the level of VRK3 in HeLa. Hela cells were transfected with Flag-Mock or Flag-RNF144a. Cell lysates were analyzed by immunoblotting. (C) Knockdown of RNF144a inhibits poly-ubiquitination of VRK3. HEK293A cells were transfected with indicated siRNA(siRNF144a#1 or siCon) and vectors. Transfected cells were treated with 10μM MG132 for 4 hours. The cell lysates were immunoprecipitated with anti-Flag antibody and VRK3 poly-ubiquitination was analyzed by immunoblotting. (D, E) RNF144a C20A/C23A and C198A mutants were defective in promoting VRK3 ubiquitination. HEK293A cells were transfected with indicated vectors. After 24 hour incubation, cells were treated with 10μM MG132 for 6 hours. Cell lysates were immunoprecipitated with anti-Flag antibody and VRK3 poly-ubiquitination was analyzed by immunoblotting.

Figure. S4

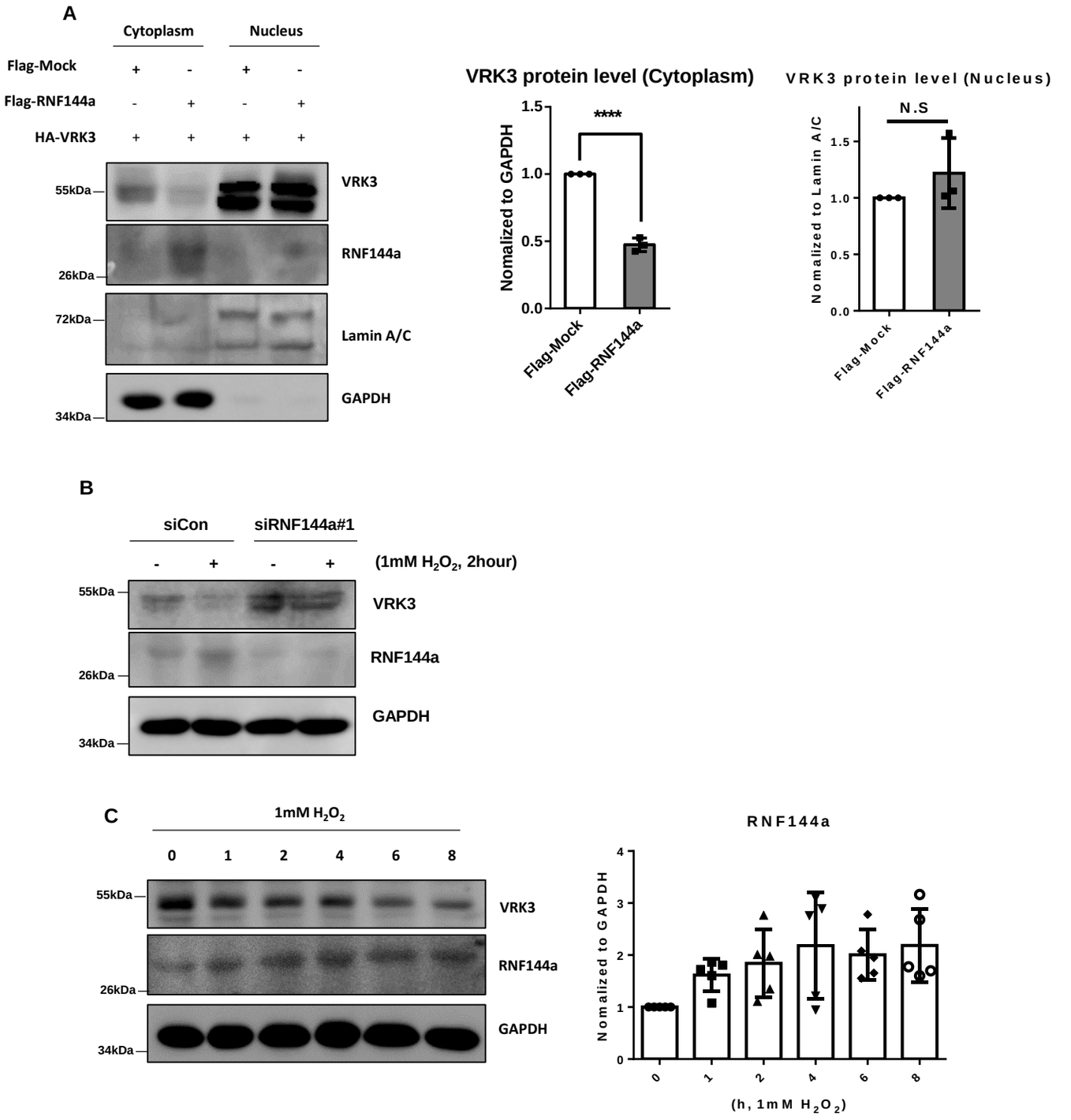

Figure S4. (A) RNF144a promotes the degradation of VRK3 located in the cytoplasm. U2OS cells were transfected with indicated vectors. Cell lysates were analyzed in nucleoplasmic and cytoplasmic fraction. Values represent means ± standard deviation (SD), (n=3, independent experiments). ****p < 0.0001, N.S : Not significant by two-tailed unpaired t-test. (B) RNF144a is crucial for H_2O_2 mediated VRK3 degradation. U2OS cells were transfected with either siRNF144a#1 or siCon. The cell lysates were analyzed by immunoblotting. (C) RNF144a expression under oxidative stress. U2OS cells were treated with 1mM H_2O_2 for indicated time. Cell lysates were analyzed by immunoblotting. VRK3 levels were normalized to GAPDH. Values represent means ± standard deviation (SD), (n=5, independent experiments). Tukey's multiple comparison test.

Figure. S5

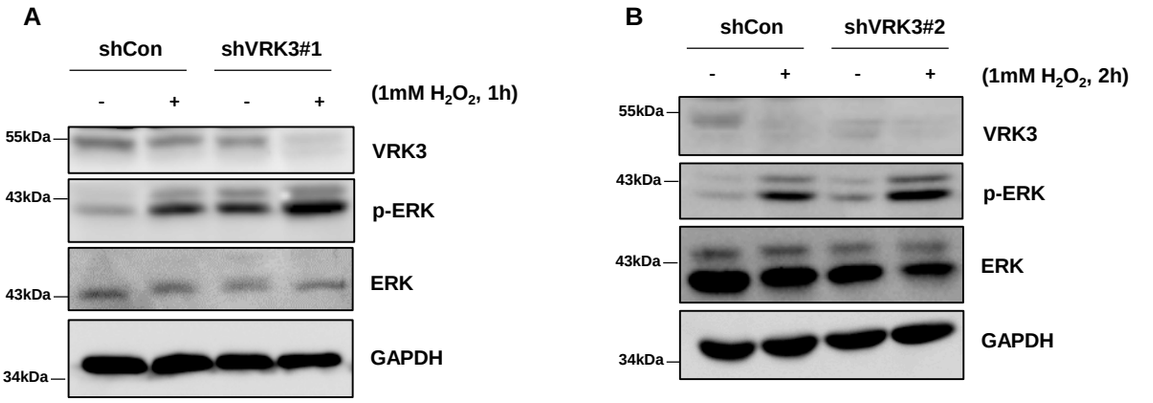

Figure S5. Knockdown of VRK3 increases the activity of ERK. (A, B) U2OS cells were transfected with either shCon or siVRK3#1(A) / siVRK3#2(B). Cells were treated with 1mM H_2O_2 for indicated times. Cell lysates were analyzed by immunoblotting.

Figure. S6

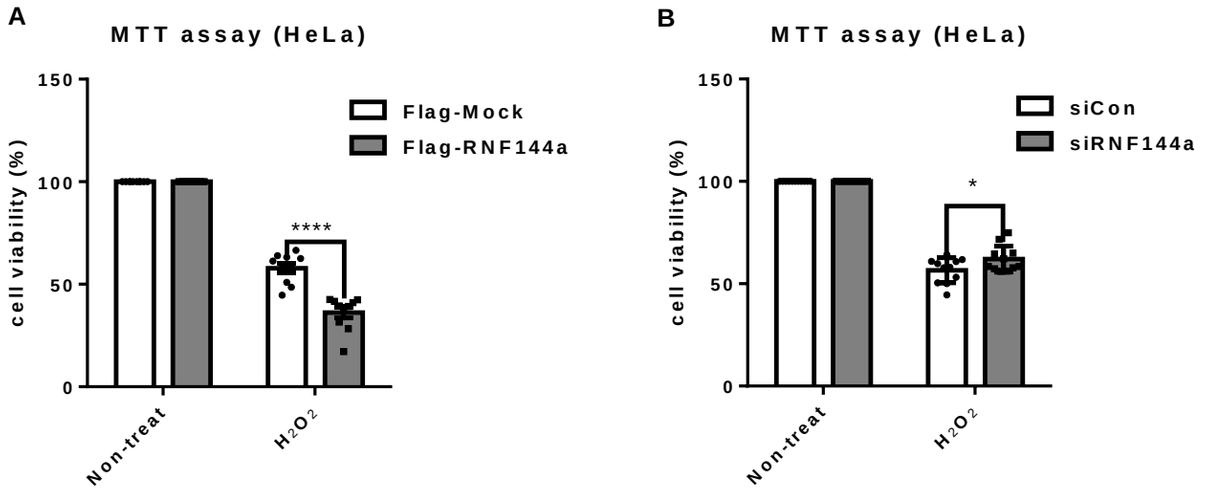

Figure S6. RNF144a induces the ERK-dependent apoptosis under oxidative stress. (A, B) HeLa cells were transfected with either Flag-Mock or Flag-RNF144a (A) / siCon or siRNF144a#2 (B) . Cells were treated with 1mM H_2O_2 for 6 hours and cell viability was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). (n=10) *p<0.05, ****p<0.0001 by Tukey's multiple comparison test.

Table. S1

siRNA sequence

All siRNAs were purchased from Bioneer

siCon	5’-CCUACGCCACCAAUUUCGU(dtdt)-3’
siVRK3	5’-GGACAAAUUGCCUUCCCAA(dTdT)-3’
siRNF144a#1	5’-GAUGAUUUCCUUCUGAUAC(dTdT)-3’
siRNF144a#2	5’-CAGUAUGUUGAGCUCUUGA(dTdT)-3’

siRNA sequence

The pLL3.7 LentiLox system was used to generate shRNA.

shVRK3#1	5’-ACTCAAGGCCTGCTGTTA-3’
shVRK3#2	5’-TGAGTTCATTAGCATGGAC-3’