Supplementary Figure Legends

Figure S1: VHL renders renal cancer cell resistant to cystine deprivation-induced cell death

(A, B). Crystal violet staining of RCC4 (A) or 786-O (B) cells expressing either empty vector (Vec) or VHL protein after 24 hours deprivation of indicated individual or all (AA) amino acids.

(C). Cell morphology of 786-O Vec and VHL cells after 16 hours deprivation of cystine.

(D). Crystal violet staining of RCC4 and 786-O expressing either empty vector or VHL cells after 48 hours exposure to different level of cystine deprivation.

(E). Crystal violet staining of RCC4 Vec and VHL cells after 48 hours treatment of indicated concentrations of sulfasalazine (SAS).

(F). Cell numbers of 786-O Vec and VHL cells after treatment with indicated concentrations of BSO and SAS for 2 days. Crystal violet staining of 786-O Vec and VHL cells 2 days after combination treatment of BSO and SAS.

(G). Western blot analysis of VHL protein expression in one patient-derived primary ccRCC tumor cells as well as *VHL*-deficient and *VHL*-restored RCC cells.

Figure S2: Cystine deprivation induces a programmed necrosis in *VHL*-deficient renal cancer cells

(A). The percentage of RCC4 cell population stained by propidium iodide (PI) and annexin-V upon control, cystine deprivation or etopside (12.5 μM) treatments at indicated times.

(B). Crystal violet staining of RCC4 cells under cystine deprivation that are also treated with the pan-caspase inhibitor (Z-Vad, 20 μM) or the necrosis inhibitor necrostatin-1 (Nec-1, 10 μM) for 2 days.

(C). Cell cytotoxicity of the patient-derived primary ccRCC cells after 24 hours cystine deprivation or combined treatment with Nec-1.

(D). Crystal violet staining of 786-O cells after 24 hours cystine deprivation or combined treatment with the MLKL inhibitor NSA.

(E). Western blot analysis of MLKL oligomerization by two different MLKL phosphorylation antibodies (pho-S357 or pho-S358) in 786-O cells after 18 hours of cystine deprivation or co-treatment with indicated concentrations of NSA. Protein samples were prepared in a reducing (+ β-ME) condition.

(F). Western blot analysis of MLKL oligomerization and monomer by two different MLKL phosphorylation antibody (pho-S357 or pho-S358) in 786-O Vec and VHL cells after 18 hours cystine deprivation.

Figure S3: VHL represses TNFα expression to protect from Cystine-deprived necrosis

(A) Cell number of 786-O Vec or two shHIF-2α cells after exposing to 200 or 1 μM of cystine for 24 hours. Immunoblotting of HIF-2α protein indicated the efficient knockdown by both shRNAs.

(B) Crystal violet staining of 786-O Vec and two different shHIF-2α cells after 48 hours of exposure to indicated levels of cystine.

(C). Relative mRNA level of TNFα in Vec or VHL infected RCC4 and 786-O cells by qPCR.

(D). Western blot analysis of RIPK1, PARP1, and Casp8 cleavage in 786-O cells after 3 days of viral infection with scramble or two shRNAs against RIPK1.

(E). Analysis of PARP1 and caspase-8 (Casp8) cleavage in RCC4 Vec and VHL cells infected with shRIPK1 or control virus, or without infection at day 5.

(F). Cell cytotoxicity (upper panel) and crystal violet staining (lower panel) of RCC4 shScr and two different shTNFα cells after 24 hours of cystine deprivation.

(G). Immunoblotting of MLKL oligomerization by pho-MLKL-S357 antibody and TNFα protein expression under reducing (+ β-ME) condition in RCC4 infected by shScr and two different shTNFα.

Figure S4: Metabolomic and transcriptional response to cystine deprivation

(A). Relative levels of 158 intracellular metabolites (mean-centered and clustered) in 786-O Vec or VHL cells after 18 hours exposure to high (HC, 200 μM) or low cystine (LC, 1 μM).

(B). Zoom view of a set of increased metabolites under cystine deprivation in ((A), blue bar).

(C). Cell cytotoxicity (upper panel) and crystal violet staining (lower panel) of RCC4 shScr and two shNoxa cells after 24 hours exposure to indicated levels of cystine.

(D). Immunoblotting of Noxa protein expression in RCC4 shScr and two shNoxa cells after 18 hours exposure to indicated cystine concentrations.

Figure S5: Activation of Src and p38 kinases is required for cystine-deprived necrosis

(A). Cell viability of 786-O cells under high (HC, 200 μM) or low cystine (LC, 1 μM) in the presence or absence of NADPH oxidase inhibitor VAS-2870 (VAS), JNK inhibitor SP600125 (SP), p38 MAPK inhibitor SB203580 (SB) or PI3K inhibitor LY294002 (LY) at indicated concentrations.

(B). Crystal violet staining of RCC4 cells under cystine deprivation in the presence or absence of p38 MAPK inhibitor SB203580 for 2 days treatments.

(C). Immunoblotting of p-p38 and Noxa protein expression in RCC4 and 786-O Vec or VHL cells under indicated levels of cystine.

(D). Crystal violet staining of 786-O cells under cystine deprivation with Src inhibitor (Su6656) and FAK inhibitor (PF-573228) for 24 hrs.

(E). Western blots analysis of Src protein in 786-O cells transfected with siCon, individual or pooled siSrcs.

(F) Western blots analysis of Src and different protein modifications in the Src antibody immuno-precipitates (Src IP) and whole cell lysates (WCL) of 786-O Vec and VHL cells treated with indicated cystine level for 18 hours.

Figure S6: Mutual amplification between the Src-p38-Noxa and TNF-RIP1/3-MLKL pathways

(A). Cell cytotoxicity of RCC4 cells after 24 hours of cystine deprivation in the presence or absence of rotenone at indicated concentrations.

(B). Cell cytotoxicity of 786-O after 24 hours of cystine deprivation in the presence or absence of rotenone, Nec-5, SB and Su6656 at indicated concentrations.

(C). Immunoblotting of pho-p38 and Noxa protein expression in RCC4 shScr and shNoxa cells after 16 hours cystine deprivation.

(D, E). R-value projection analysis of TNFα gene signature (TNFα-Sig, (D)) and Cystine deprived gene signature (CysDep-Sig, (E)) on the gene expression dataset of *VHL* deficient and *VHL*-restored RCC4 cells.

(F, G). R-value projection analysis of TNFα-Sig (F) and CysDep-Sig (G) on the gene expression dataset (GSE36895) of paired renal normal tissue and ccRCC. The *coefficients* were presented with either normal or tumor tissue groups.

(H). R-value projection analysis of CysDep, TNFα and VHL gene signature on renal clear cell tumor dataset (606 RCC tumors) from TCGA data portal.

Figure S7: Low doses of erastin share a similar mechanism as cystine deprivation to trigger programmed necrosis in ccRCC

(A). Cell viability by crystal violet staining (2 days) of 786-O Vec, VHL and different shRNA sub-lines cells treated with indicated concentration of erastin.

(B). Western blots analysis of Noxa protein induction in 786-O Vec and VHL cells treated with 5 μM erastin for 3 or 6 hours.

(C). Cell viability (2 days) of RCC4 shScr and shRIPK1 cells treated with 2 μM erastin.

(D). Cell viability (2 days) of RCC4 cells treated with 0.5 mM sulfasalazine in the presence indicated concentrations of either Nec-1 or ferrostatin-1 (Fer-1) inhibitor.