

Supplementary Information

Fig.S1 TNF- α autocrine is dispensable for IFN- γ -induced cell death.

(a) The indicated knockout L929 cell lines were treated with DMSO (ctrl), IFN- γ , IFN- γ +GSK872 (10 μ M) for 36h and cell survival was determined by measuring ATP levels. (**b-d**) TOP: The indicated knockout L929 cell lines were treated with DMSO (ctrl), zVAD, IFN- γ , IFN- γ +zVAD or TNF for 36h and cell survival was determined by measuring ATP levels. Bottom: RIPK1, FADD, Caspase-8, GAPDH levels in the indicated knockout L929 cell lines were determined by Western blot. For **a-d**, data are from n = 3 independent experiments. Error bars represent mean \pm s.e.m. of n = 3 independent experiments. Two-tailed Student's t-test was applied for P values; * $P < 0.05$, ** $P < 0.01$. For **b-d** (down), results shown are representative of three independent experiments.

Fig.S2 PKR is not required for IFN- γ -induced cell death.

(a-c) TOP: The indicated knockout L929 cell lines were treated with DMSO (ctrl), zVAD, IFN- γ , IFN- γ +zVAD for 36h and cell survival was determined by measuring ATP levels. Bottom: RIPK1, PKR, GAPDH levels in the indicated knockout L929 cell lines were determined by Western blot. For **a-c**, data are from n = 3 independent experiments. Error bars represent mean \pm s.e.m. of n = 3 independent experiments. Two-tailed Student's t-test was applied for P values; ** $P < 0.01$. For **a-c** (down), results shown are representative of three independent experiments.

Fig. S3 Flag-RIPK3 reconstitution restores IFN-induced necroptosis.

(a)The indicated knockout L929 cell lines that were infected with lentivirus expressing vector encoding nothing, or FLAG-RIPK3. Then these cells were treated with DMSO (ctrl) zVAD, IFN- γ or IFN- γ +zVAD for 36h. Cell survival was determined by measuring ATP levels. Two-tailed Student's t-test was applied for P values; ** $P < 0.01$. (b) RIPK3, GAPDH protein levels in the cells described above were determined by Western blot.

Fig. S4 Overexpression of ZBP1 and dimerization of ZBP1-CD induces cell death in WT L929 cells.

(a) Left: WT L929 cell lines that were infected with gradually increased dose of lentivirus expressing vector encoding FLAG-ZBP1. L929 cells infected with empty vector and treated with nothing (-) or IFN- γ for 36h were included as control. Cell survival was determined by measuring ATP levels (Left panel); ZBP1, GAPDH protein levels were determined by Western blot (Right panel). **(b)** WT L929 cell lines that were infected with lentivirus expressing vector encoding FLAG-HBD-CD, and then treated with ethanol (Ctrl), 4-OHT. L929 cells infected with empty vector and treated with nothing (-) or IFN- γ for 36h were included as control. Cell survival was determined by measuring ATP levels (Left panel). ZBP1, HBD-CD, GAPDH protein levels were determined by Western blot. Cell survival data are from n = 3 independent experiments. Error bars represent mean \pm s.e.m. of n = 3 independent experiments. Western blot results shown are representative of three independent experiments.

Fig. S5 The role of RIPK1 kinase activity and RIPK1's association with JAK1-STAT1 in IFN γ -induced necroptosis

(a) The FADD KO and Caspase-8 KO L929 cell lines were treated with DMSO, Nec-1(30 μ M), IFN- γ , IFN- γ +Nec-1 for 36h and the cell survival was determined by measuring ATP levels. **(b)** 293T cells were transfected with the plasmids as indicated. Total cell lysates and anti-FLAG immunoprecipitates were immunoblotted to detect the indicated proteins. For **a**, data are from n = 3 independent experiments. Error bars represent mean \pm s.e.m. of n = 3 independent experiments. For **b**, results shown are representative of three independent experiments.

Fig. S6 The generation and validation of ZBP1^{-/-} mice and various knockout cell lines.

(a) Genomic DNA sequence of ZBP1 locus in ZBP1^{-/-} mice generated by CRISPR/Cas9. **(b)** ZBP1 and GAPDH protein levels in ZBP1^{+/+} and ZBP1^{-/-} bone marrow-derived macrophages stimulated with IFN- γ and IFN- β . **(c)** Age- and sex-matched WT and ZBP1 KO mice were injected with TNF through the tail vein, respectively. N=6 mice for each group, data are pooled from two independent

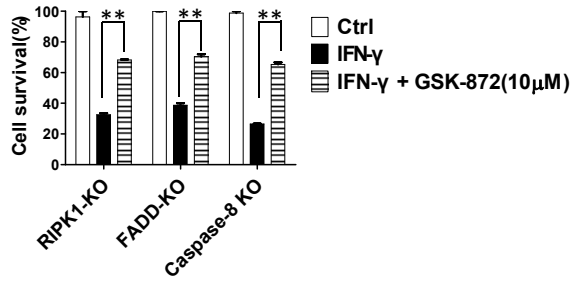
experiments. **(d)** Genomic DNA sequences of *FADD* locus in *FADD* KO L929 cells. **(e)** Genomic DNA sequences of *Caspase-8* locus in *Caspase-8* KO L929 cells. **(f)** Genomic DNA sequences of *MLKL* locus in *RIPK1/MLKL* DKO L929 cells, *RIPK1/MLKL* DKO L929 cells were generated by targeting *MLKL* in *Rip1* KO L929 cells. **(g)** Genomic DNA sequences of *MLKL* locus in *FADD/MLKL* DKO L929 cells, *FADD/MLKL* DKO L929 cells were generated by targeting *MLKL* in *FADD* KO L929 cells. **(h)** Genomic DNA sequences of *RIPK3* locus in *FADD/RIPK3* DKO L929 cells, *FADD/RIPK3* DKO L929 cells were generated by targeting *RIPK3* in *FADD* KO L929 cells. **(i)** Genomic DNA sequences of *RIPK3* locus in *Caspase-8/RIPK3* DKO L929 cells, *Caspase-8/RIPK3* DKO L929 cells were generated by targeting *RIPK3* in *Caspase-8* KO L929 cells.

Fig. S7 Sequence information of knockout cell lines.

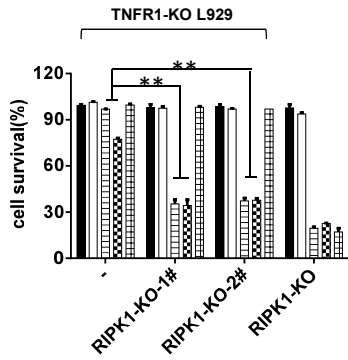
(a) Genomic DNA sequences of *ZBP1* locus in *RIPK1/ZBP1* DKO L929 cells, *RIPK1/ZBP1* DKO L929 cells were generated by targeting *ZBP1* in *RIPK1* KO L929 cells. **(b)** Genomic DNA sequences of *ZBP1* locus in *FADD/ZBP1* DKO L929 cells, *FADD/ZBP1* DKO L929 cells were generated by targeting *ZBP1* in *FADD* KO L929 cells. **(c)** Genomic DNA sequences of *RIPK1* locus in *TNFR1/RIPK1* DKO L929 cells, *TNFR1/RIPK1* DKO L929 cells were generated by targeting *RIPK1* in *TNFR1* KO L929 cells. **(d)** Genomic DNA sequences of *TNFR1* locus in *TNFR1/FADD* DKO L929 cells, *TNFR1/FADD* DKO L929 cells were generated by targeting *TNFR1* in *FADD* KO L929 cells. **(e)** Genomic DNA sequences of *Caspase-8* locus in *TNFR1/Caspase-8* DKO L929 cells, *TNFR1/Caspase-8* DKO L929 cells were generated by targeting *Caspase-8* in *TNFR1* KO L929 cells. **(f)** Genomic DNA sequences of *PKR* locus in *PKR* KO L929 cells. **(g)** Genomic DNA sequences of *PKR* locus in *FADD/PKR* DKO L929 cells, *FADD/PKR* DKO L929 cells were generated by targeting *PKR* in *FADD* KO L929 cells. **(h)** Genomic DNA sequences of *PKR* locus in *Caspase-8/PKR* DKO L929 cells, *Caspase-8/PKR* DKO L929 cells were generated by targeting *PKR* in *Caspase-8* KO L929 cells. **(i)** Genomic DNA sequences of *RIPK1* locus in *PKR/RIPK1* DKO L929 cells, *PKR/RIPK1* DKO L929 cells were generated by targeting *RIPK1* in *PKR* KO L929 cells.

Figure S1

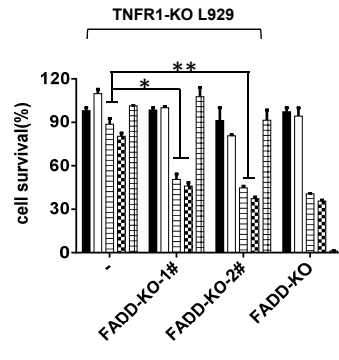
a



b



c



d

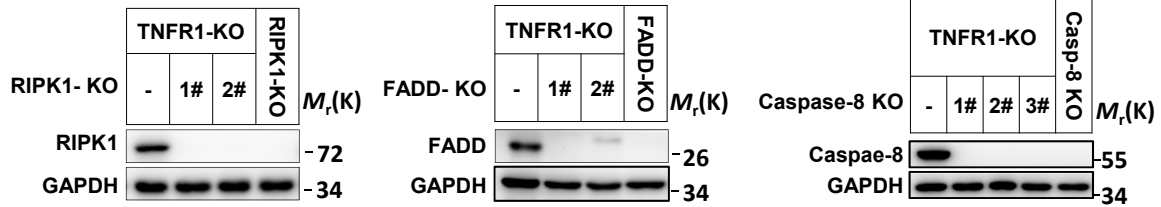
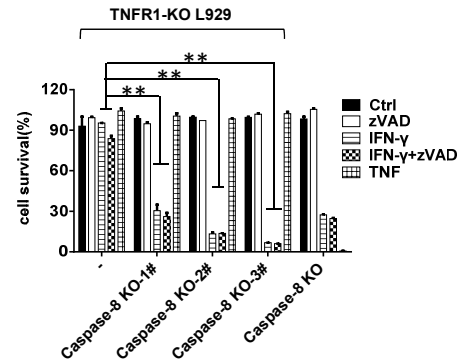


Figure S2

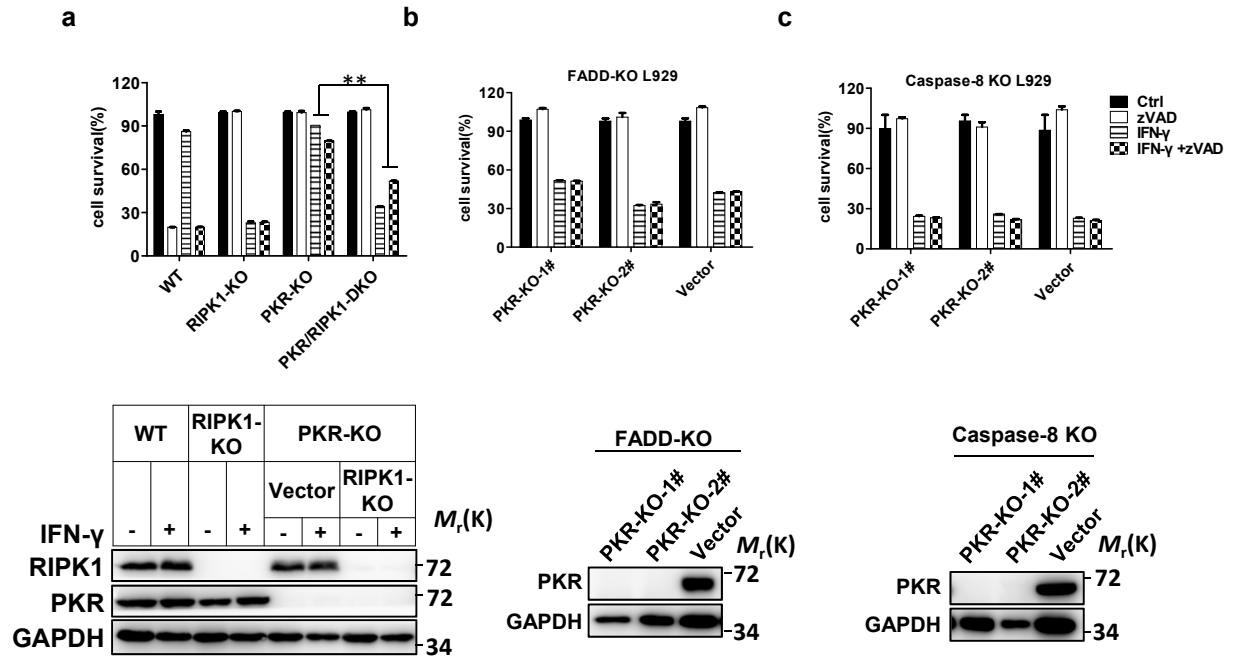


Figure S3

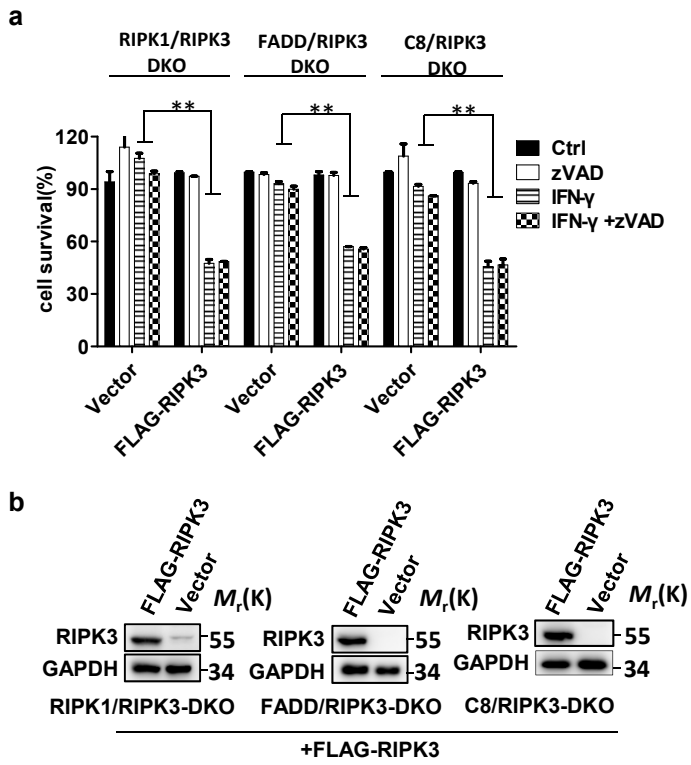
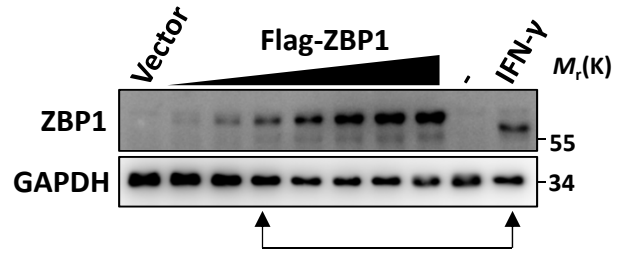
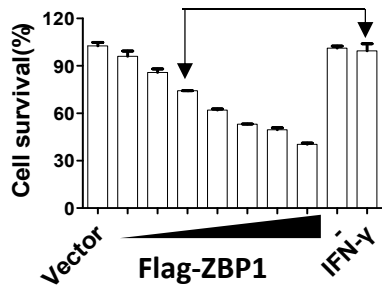


Figure S4

a



b

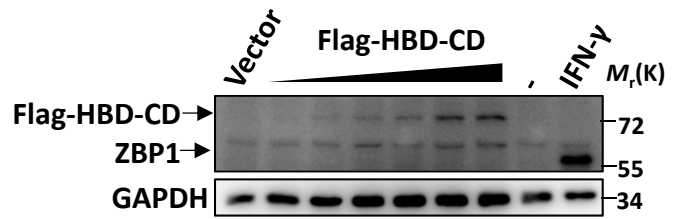
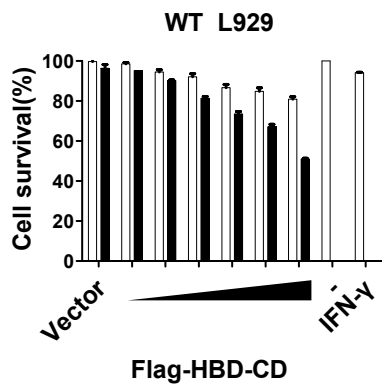
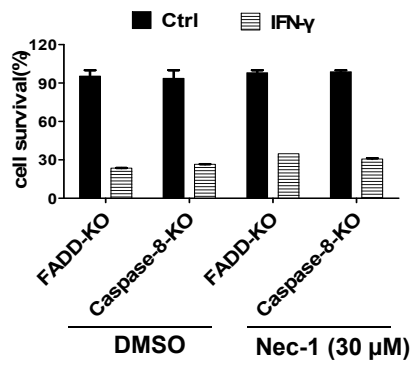


Figure S5

a



b

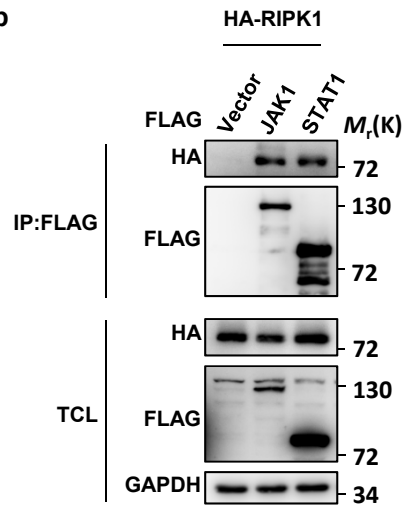
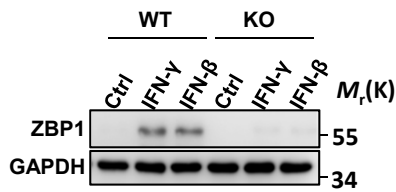


Figure S6

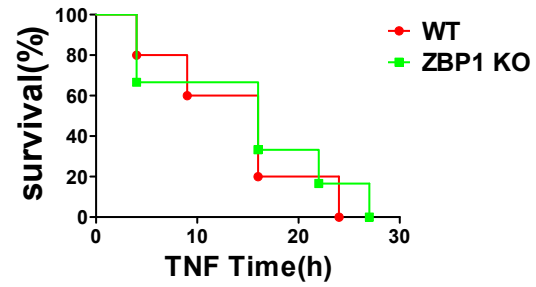
a

WT **GCTATGACGGACAGACGTGGAAGATCTACCACTCA**CGTCA**GGAAGGCCAAGACATAG**
 KO **GCTATGACGGACAGACGTGGAAGATTTACCACTCA**-----**GGAAGGCCAAGACATAG**

b



c



d

FADD-KO
 FADD-wt **TCAAGTTCCTGTGCGCG****CGAGCGCGTGAGCAAACGAAAGCTGGAGCGCG**
 FADD KO-allele-1 **TCAAGTTCCTGTGCGCG**-----**GAGCGCGTGAGCAAACGAAAGCTGGAGCGCG**
 FADD KO-allele-2 **TCAAGTTCCTGTGCGCG**-----**CGCGCGTGAGCAAACGAAAGCTGGAGCGCG**
 FADD KO-allele-3 **TCAAGTTCCTGTGCGCG**-----**GACCGCGTGA****CAAACA****AAAGCTGGAGCGCG**

e

Caspase-8 KO
 Caspase-8-wt **TTTCCACATCAGTCGGTGGGACCTGCTGG**-----**TCAACTTCCTAGAC**
 Caspase-8 KO-allele-1 **TTTCCACATCAG**-----**GTCAACTTCCTAGAC**
 Caspase-8 KO-allele-2 **TTTCCACATCAGTCGGTGGGACCTGCTGGGT****CAACTTCCTAGAC**
 Caspase-8 KO-allele-3 **TTTCCACATCAGTCGGTGGGACCTGCT**-----**CAACTTCCTAGAC**

f

RIPK1/MLKL-DKO
 MLKL-wt **ATTGGAATACCGTTTCAGATGTCAGCCAGCCAGCATCCTGGCA**
 MLKL KO-allele-1 **ATTGGAAT**-----**G**-----**CCAGCATCCTGGCA**
 MLKL KO-allele-2 **ATTGGAATACCGTTTCAG**-----**CCAGCATCCTGGCA**
 MLKL KO-allele-3 **ATTGGAATACCGTTT**-----**CCACATCCTGGCA**

g

FADD/MLKL-DKO
 MLKL-wt **GGTTTATCATTGGAATACCGTTTCAGATGTCAGCCAGCCAGCATCCTGGCA****GCAGCAGGAAGATC**
 MLKL KO-allele-1 **GGTTTATCATTGGAATACCGTTTCAG**-----**A****CATCCTGGCA****CCGGCAGGAAGATC**
 MLKL KO-allele-2 **GGTTTATCATTGGAAT**-----**G**-----**CCAGCATCCTGGCA****GCAGCAGGAAGATC**
 MLKL KO-allele-3 **GGTTTATCATTGGAATACCGTTTCAG**-----**CCAGCATCCTGGCA****GCAGCAGGAAGATC**

h

FADD/RIPK3-DKO
 RIPK3-wt **ATTCTGCTGGATCCAGAGCTCCACGCCAAGGTTAGT**
 RIPK3 KO-allele-1 **ATTCTGCTGGATCCCA**-----**CCCGCCAAGGTTAGT**
 RIPK3 KO-allele-2 **ATTCTGCTGGATCCA**-----**CGCCAAGGTTAGT**
 RIPK3 KO-allele-3 **ATTCTGCTGGATCCAG**-----**CTCGCCAAGGTTAGT**

i

Caspase-8/RIPK3-DKO
 RIPK3-wt **ATTCTGCTGGATCCAGAGCTCCACGCCAAGGTTAGTCCA**
 RIPK3 KO-allele-1 **ATTCTGCTGGATCCAGAGC**-----**CACGCCAAGGTTAGTCCA**
 RIPK3 KO-allele-2 **ATTCTGCTGGATCCCA**-----**CCAAGGTTAGTCCA**
 RIPK3 KO-allele-3 **ATTCTGCTGGATCCAGAGC**-----**CCCGCCAAGGTTAGTCCA**

Figure S7

a

RIPK1/ZBP1-DKO

ZBP1-wt TGACGGACAGACGTGGAGATCTAC CACTCACGTCAGGAAGGCCAAGACATAGGTATGAC
 ZBP1 KO-allele-1 TGACGGACAGACGTGGAGAGAC-----ATAGGTATGAC
 ZBP1 KO-allele-2 TGACGGACAGACGT-----ATATAC-----ATAGGTATGAC
 ZBP1 KO-allele-3 TGACGGACAGAC-----ATAGGTATGAC

b

FADD/ZBP1-DKO

ZBP1-wt GATCTACCACTCAC-CTCAGGAGGCCAAGACATA
 ZBP1 KO-allele-1 GATCTACCACTCC---TCAGGAGGCCAAGACATA
 ZBP1 KO-allele-2 GATCTACCACTACCCTCAGGAGGCCAAGACATA
 ZBP1 KO-allele-3 GATCTACCACTT---CTCAGGAGGCCAAGACATA

c

TNFR1/RIPK1-DKO

RIPK1-wt TAGAAGAAGGGAACATACTCGCTGGTATGGAGTACATGGAGAAGGGCAAC
 RIPK1 KO-allele-1 TAGAAGAAGGGAACATA-----TGGAGAAGGGCAAC
 RIPK1 KO-allele-2 TAGAAGAAGGGAACATACT--CTGGTATGGAGTACATGGAGAAGGGCAAC
 RIPK1 KO-allele-3 TAGAAGAAGGGAACATA--CGCTGGTATGGAGTACATGGAGAAGGGCAAC

d

TNFR1/FADD-DKO

TNFR1-wt ACCTGAGTGAGACACACTTCAGTCCGTGGACTGCAGCCCCCTGCTCAACGGCACCGTGACAATCCCCTGTGAGCACCGGTACCCC
 TNFR1 KO-allele-1 ACCTGAGTGAGACACACTTC-----C-----CTGGATGACCGGTACCCC
 TNFR1 KO-allele-2 ACCTGAGTGAGACACACTTCAGTCCGTGGACTGCAGCCCCCTGCTCAACGGCACCGTGACAATCCCCTGTGAGCACCGGTACCCC
 TNFR1 KO-allele-3 ACCTGAGTGAGACACACTTCAGTCCGTGGACTGCAGCCCCCTGCTCAACGGCACCGTGACAATCCCCTGTGAGCACCGGTACCCC

e

TNFR1/Caspase-8

Caspase-8-wt GTCGGTGGGACCTGCTGGTCAACTTCCTAGAC
 Caspase-8 KO-allele-1 GTCGGTGGGACCTGCTG---CAACTTCCTAGAC
 Caspase-8 KO-allele-2 GTCGGTGGGACCTGCTG-TCAACTTCCTAGAC
 Caspase-8 KO-allele-3 GTCGGTGGGACCTG---TCAACTTCCTAGAC

f

PKR-KO

PKR-wt AAAATGGCCAGTGA---ACCCCAAGTTTCTACATGGA
 PKR KO-allele-1 AAAATGGCCAGTGA---ACCCCAAGTTTCTACATGGA
 PKR KO-allele-2 AAAATGGCCAGTGA---ACCCCAAGTTTCTACATGGA
 PKR KO-allele-3 AAAATGGCCAGTGA-TACCCCAAGTTTCTACATGGA

g

FADD/PKR-DKO

PKR-wt AGGCCACTGGGAGGAAAAATGGCCAGTGA-ACCCCAAGTTTCTA
 PKR KO-allele-1 AGGCCACTGGCCATTTTCTCTCCAGTGGCTGAAAGTTTCTA
 PKR KO-allele-2 AGGCCACTGGGAGGAAA-----AATGGC-----AGTTTCTA
 PKR KO-allele-3 AGGCCACTGGGAGGAAAAATGGCCAGTGA-ACCCCAAGTTTCTA

h

Caspase-8/PKR-DKO

PKR-wt AAAATGGCCAGTGA-TACCCCAAGTTTCTACATGGACAAA
 PKR KO-allele-1 AAAATGGCCAGT---ATACCCAAGTTTCTACATGGACAAA
 PKR KO-allele-2 AAAATGGCCAGTGA---CCCAAGTTTCTACATGGACAAA
 PKR KO-allele-3 AAAATGGCCAGTGAATACCCAAGTTTCTACATGGACAAA

i

PKR/RIPK1-DKO

RIPK1-wt CATCATAGAAGAGGGAACATAT-----CGCTGGT-----ATGGAGTACATGGAGAAGGG
 RIPK1 KO-allele-1 CATCATAGAAGAGGGAACATATGATGTGATGATGGTGTGTGATGGAGACATGGAGAAGGG
 RIPK1 KO-allele-2 CATCATAGAAGAGGGAACATAT-----CGCTGGT-----ATGGAGTACATGGAGAAGGG
 RIPK1 KO-allele-3 CATCATAGAAGAGT-----CCATGGAGTACATGGAGAAGGG