

Supplemental information

**Whole-genome CRISPR screen identifies
RAVER1 as a key regulator of RIPK1-mediated
inflammatory cell death, PAnoptosis**

R.K. Subbarao Malireddi, Ratnakar R. Bynigeri, Raghvendra Mall, Eswar Kumar Nadendla, Jon P. Connelly, Shondra M. Pruett-Miller, and Thirumala-Devi Kanneganti

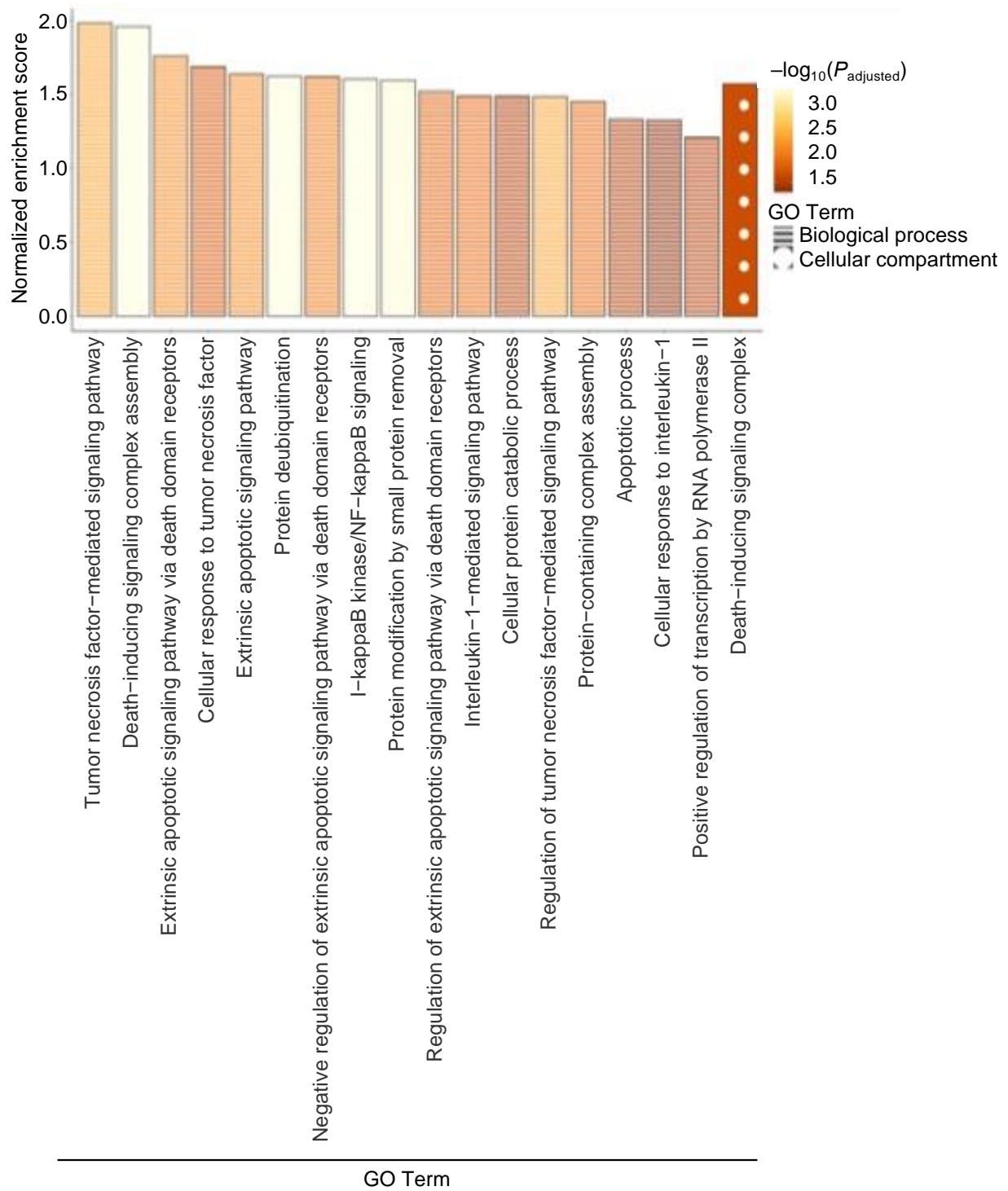


Figure S1. Cell signaling processes and complexes are enriched in the TAK1 inhibitor CRISPR screen. See also Figure 1

Top enriched Gene Ontology (GO) terms among all positively enriched gRNA-targeted genes, including significantly enriched biological processes and cellular components, using the 'fgsea' package in R are shown.

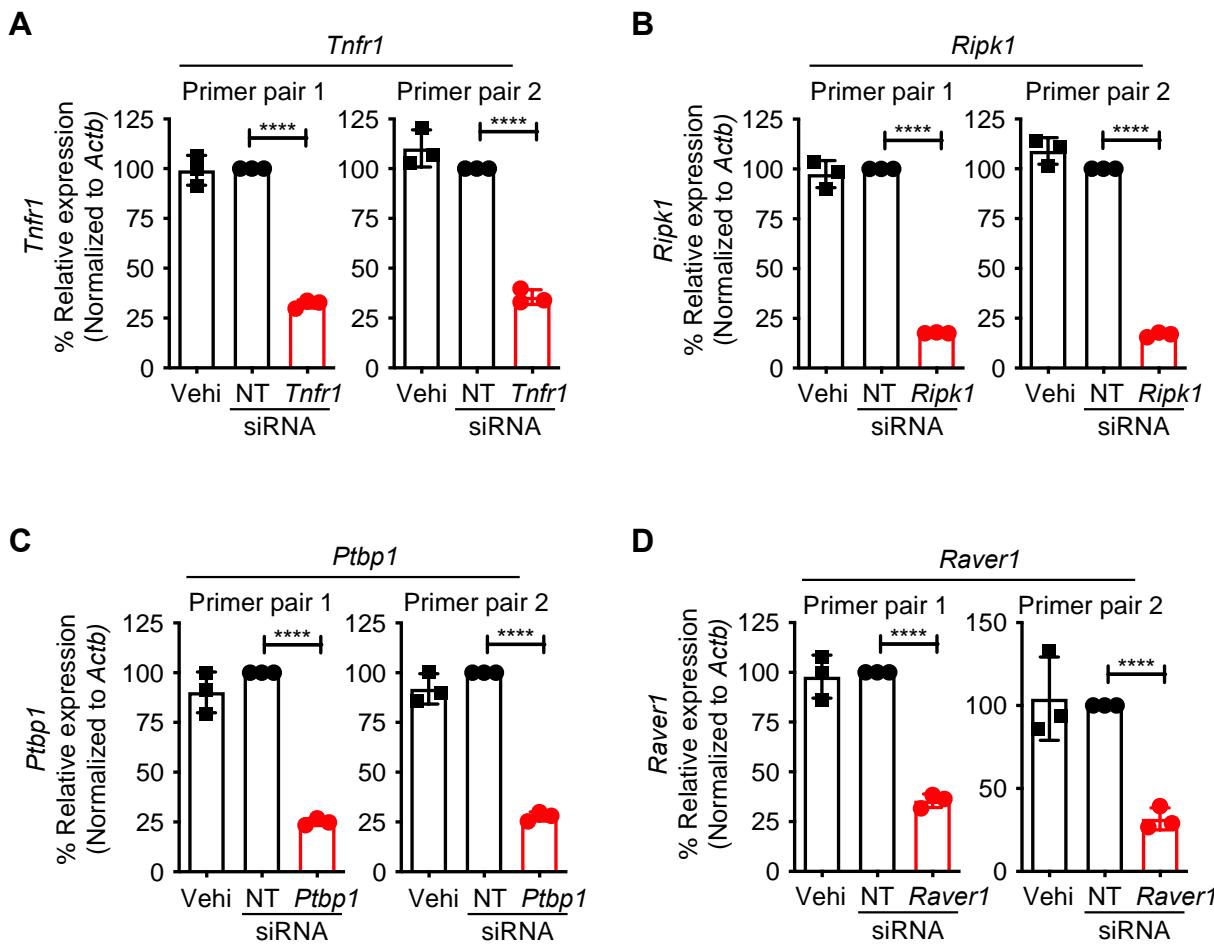


Figure S2. siRNA successfully knocked down the gene expression of *Tnfr1*, *Ripk1*, *Ptbp1* and *Raver1*. See also Figure 2

(A–D) The indicated qPCR analyses were performed from the cDNA prepared from the total RNA isolated from bone marrow-derived macrophages (BMDMs) treated with vehicle control (Vehi) or transfected with NT (non-targeting) siRNA or gene-specific siRNAs for 48 h. The data were normalized to the internal control of *Actb* mRNA expression and presented as percent knockdown with respect to the NT siRNA transfected BMDMs. All data are presented as mean \pm SEM, from three replicate samples ($n = 3$) (A–D). $P < 0.05$ is considered statistically significant. **** $P < 0.001$ (two-tailed *t* test [B]). Data are representative of three independent experiments (A–D).

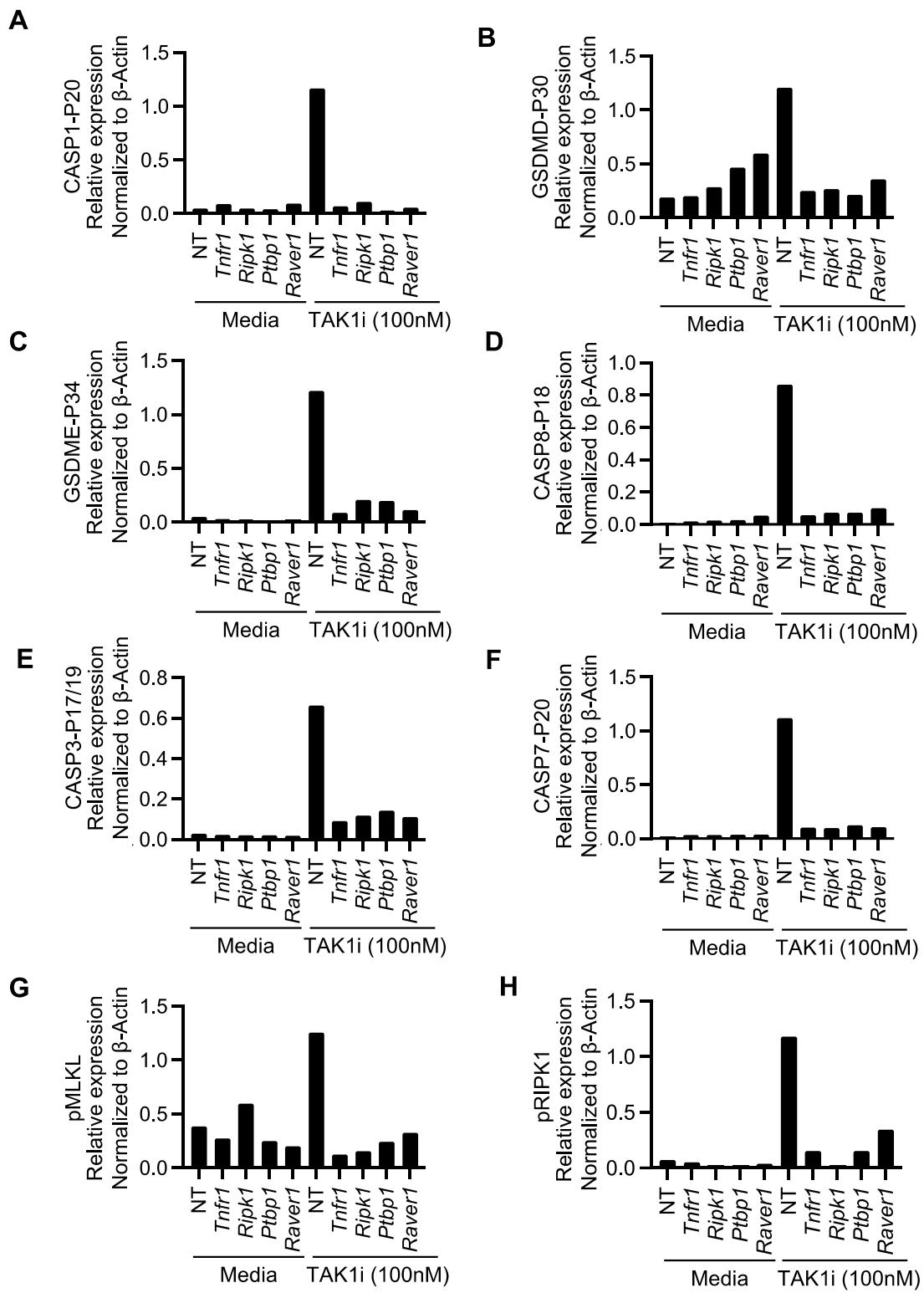


Figure S3. TAK1 inhibition-mediated PANoptosis is blocked by loss of PTB splicing factors. See also Figure 3

(A–H) Densitometry quantification of Western blot data reported in Figure 3. Densitometry expressed as relative values normalized to β -Actin expression. Data are representative of three independent experiments with $n = 2\text{--}3$ in each repeat (A–H).

A

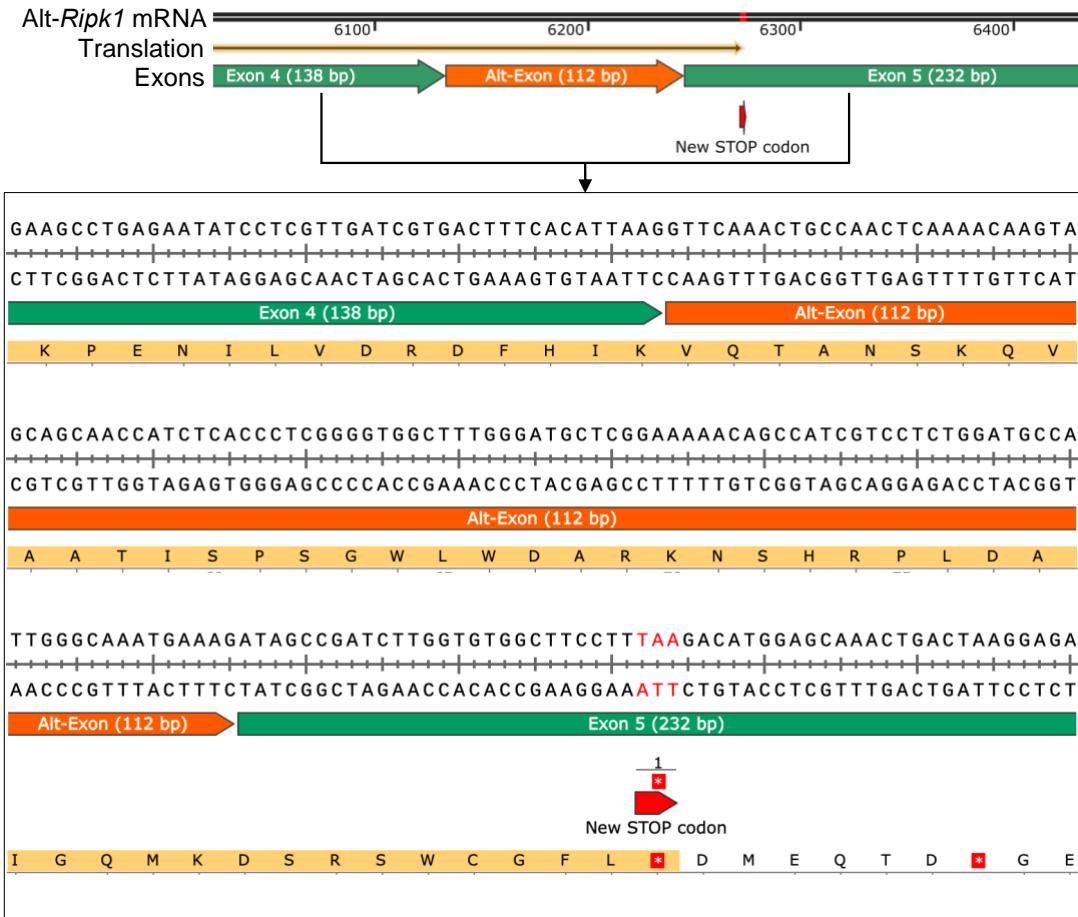
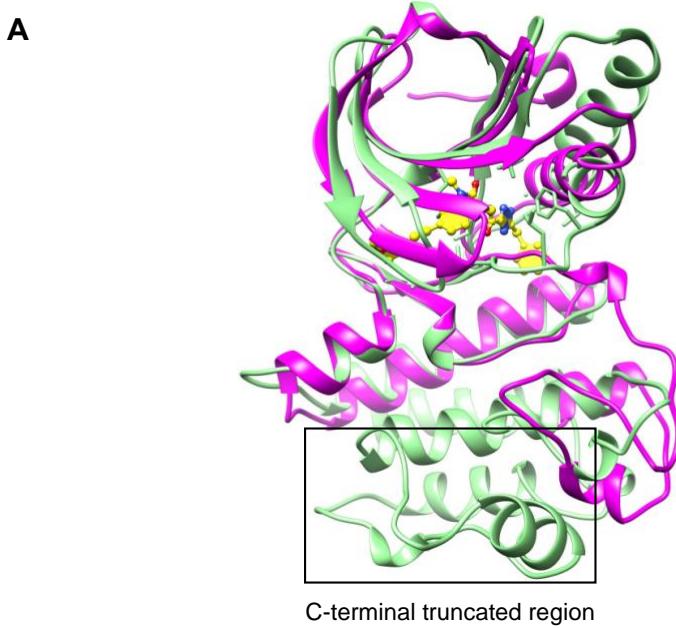


Figure S4. The alternative *Ripk1* transcript contains a stop codon that results in a non-functional RIPK1. See also Figure 4

(A) Depiction of the alternative (Alt) *Ripk1* mRNA that results from alternative splicing to bring together Exon 4, Alt Exon and Exon 5. Nucleic acid base and translation details are provided, along with the Alt-exon-produced frameshift and the resulting stop codon in the *Ripk1* mRNA transcript.



B

Consensus	1	11	21	31	41
Conservation	- - - m q p d m s l	- - i k m - s s d -	l e - - d L D S G G	F G K V S L c y H R	s h G f v I I K k V
HsRIPK1-KD	G A S M Q P D M S L	N V I K M K S S D F	L E S A E L D S G G	F G K V S L A F H R	T Q G L M I M K T V
mmRIPK1-KD	- - - M Q P D M S L	D N I K M A S S D L	L E K T D L D S G G	F G K V S L C Y H R	S H G F V I L K K V
mmRIPK1-X3	- - - - -	- - - - -	D L D S G G	F G K V S L C Y H R	S H G F V I L K K V
Consensus	51	61	71	81	91
Conservation	Y t G P N r a E y N	E v L L E E g K M M	h R L R H S R V V K	U L G i I I E E G n	Y S L V M E Y M E K
HsRIPK1-KD	Y K G P N C I E H N	E A L L E E A K M M	N R L R H S R V V K	U L G V I I I E E G K	Y S L V M E Y M E K
mmRIPK1-KD	Y T G P N R A E Y N	E V L L E E G K M M	H R L R H S R V V K	U L G I I I E E G N	Y S L V M E Y M E K
mmRIPK1-X3	Y T G P N R A E Y N	E V L L E E G K M M	H R L R H S R V V K	U L G I I I E E G N	Y S L V M E Y M E K
Consensus	101	111	121	131	141
Conservation	G N L M H V L K t q	i d v P L S I K G R	I I v E a I E G M c	Y L H d K G V I H K	D L K P E N I L V D
HsRIPK1-KD	G N L M H V L K A E	M S T P L S V K G R	I I L E I I E G M A	Y L H G K G V I H K	D L K P E N I L V D
mmRIPK1-KD	G N L M H V L K T Q	I D V P L S L K G R	I I V E A I E G M C	Y L H D K G V I H K	D L K P E N I L V D
mmRIPK1-X3	G N L M H V L K T Q	I D V P L S L K G R	I I V E A I E G M C	Y L H D K G V I H K	D L K P E N I L V D
Consensus	151	161	171	181	191
Conservation	r D F H I K I a d l	g s a s f - - - - -	- k m W s k l a k e	k - n k q r e v d a	t a - k k n g g t l
HsRIPK1-KD	N D F H I K I A D L	G L A S F - - - - -	- K M W S K L N N E	E H N E L R E V D G	T A - K K N G G T L
mmRIPK1-KD	R D F H I K I A D L	G V A S F - - - - -	- K T W S K L T K E	K D N K Q K E V S S	T T K K N N G G T L
mmRIPK1-X3	R D F H I K V Q T A	N S K Q V A A T I S	P S G W L W D A R K	N - - S H R P L D A	I G Q M K D - - - - -
Consensus	201	211	221	231	241
Conservation	y y m a p e h l n d	- n a k p t e k s d	v y s f - - v l w a	i f a - k e p y e n	- i - - - q - - - - -
HsRIPK1-KD	Y Y M A P E H L N D	V N A K P T E K S D	V Y S F A V V L W A	I F A N K E P Y E N	A I A E Q Q L I M A
mmRIPK1-KD	Y Y M A P E H L N D	I N A K P T E K S D	V Y S F G I V L W A	I F A K K E P Y E N	V I C T E Q F V I C
mmRIPK1-X3	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -

Figure S5. Alternative splicing of RIPK1 results in a C-terminal truncation and structural changes. See also Figure 4

(A) The human RIPK1-KD structure (PDB ID-7FD0; green) and the alternatively spliced mouse mRIPK1-X3 variant model generated by Robetta (magenta). The C-terminal truncated region found in human RIPK1-KD that is absent in mmRIPK1-X3 is denoted by the black box. (B) Sequence alignment of human RIPK1-KD (HsRIPK1-KD), mouse RIPK1-KD (mmRIPK1-KD) and mouse alternatively spliced variant mmRIPK1-X3. The allosteric and ATP binding site residues are denoted in red vertical boxes.

Table S1. Primer sequences for qPCR analyses. See also STAR Methods

Gene	Froward primer	Seq (5' to 3')	Reverse primer	Seq (5' to 3')
<i>Tnfrsf1a</i>	Tnfrsf1a-RT-F1	CCGGGAGAAGAGGGATAGCTT	Tnfrsf1a-RT-R1	TCGGACAGTCACTCACCAAGT
<i>Tnfrsf1a</i>	Tnfrsf1a-RT-F2	GGGGATACATCCATCAGGGGT	Tnfrsf1a-RT-R2	GCTCGGACAGTCACTCACC
<i>Ripk1</i>	Ripk1-RT-F1	GAAGACAGACCTAGACAGCGG	Ripk1-RT-R1	CCAGTAGCTTACCACTCGAC
<i>Ripk1</i>	Ripk1-RT-F2	GCACCCGAACACCTGAATGA	Ripk1-RT-R2	CGAACTGCTCAGTACAGATGACA
<i>Ptbp1</i>	Ptbp1-RT-F1	CACACCCCAAAGCCTCTTTAT	Ptbp1-RT-R1	ATCTGCACAAGTGCCTCTCC
<i>Ptbp1</i>	Ptbp1-RT-F2	CAGCAGCCAATGGAAACGATA	Ptbp1-RT-R2	GCAGCTTCTGACATGGATGAC
<i>Raver1</i>	Raver1-RT-F1	ATGGCAGCTAAAGGGCTTC	Raver1-RT-R1	GCCTCGATTAAGAGCCGTGG
<i>Raver1</i>	Raver1-RT-F2	GACAGCTTTGTGACTCTGCT	Raver1-RT-R2	TCAGACTAGGTGGTAGGTTGG
<i>Ripk1-Alt-Exon</i>	Ripk1-RT-New-F1 (Forward primer in Fig. 4)	GATAATCGTGGAGGCCATAGAA	Ripk1-RT-AltExon4-R1 (Reverse primer 1 in Fig. 4)	GTGAGATGGTTGCTGCTACTTG
<i>Ripk1-Alt-Exon</i>	Ripk1-RT-New-F1 (Forward primer in Fig. 4)	GATAATCGTGGAGGCCATAGAA	Ripk1-RT-AltExon4-R2 (Reverse primer 2 in Fig. 4)	CTTCATTGCCAATGGCATCC