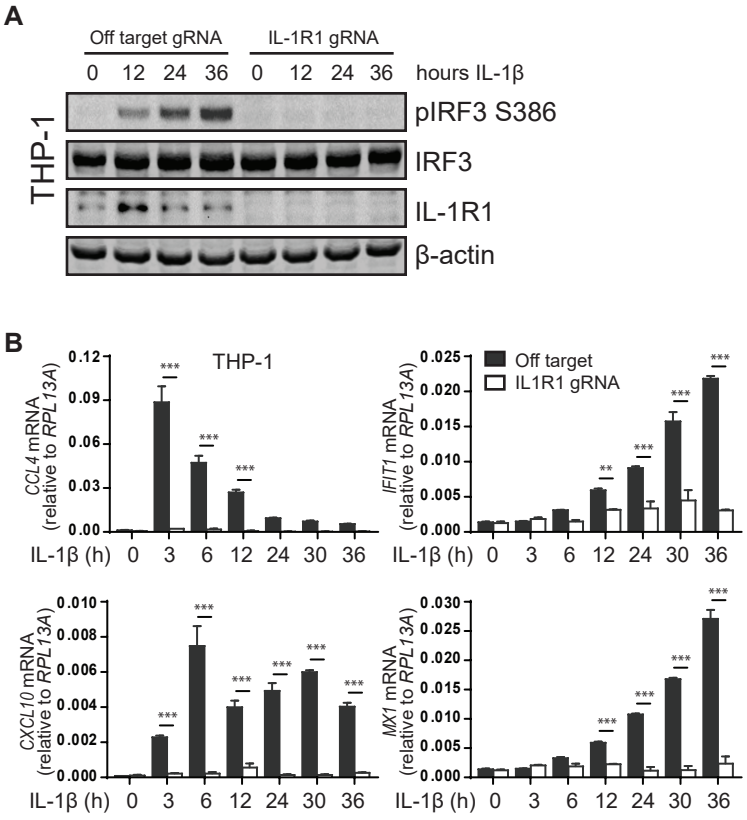
Figure S1. IL-1R1 is required for IL-1β signaling to IRF3, Related to Figure 1

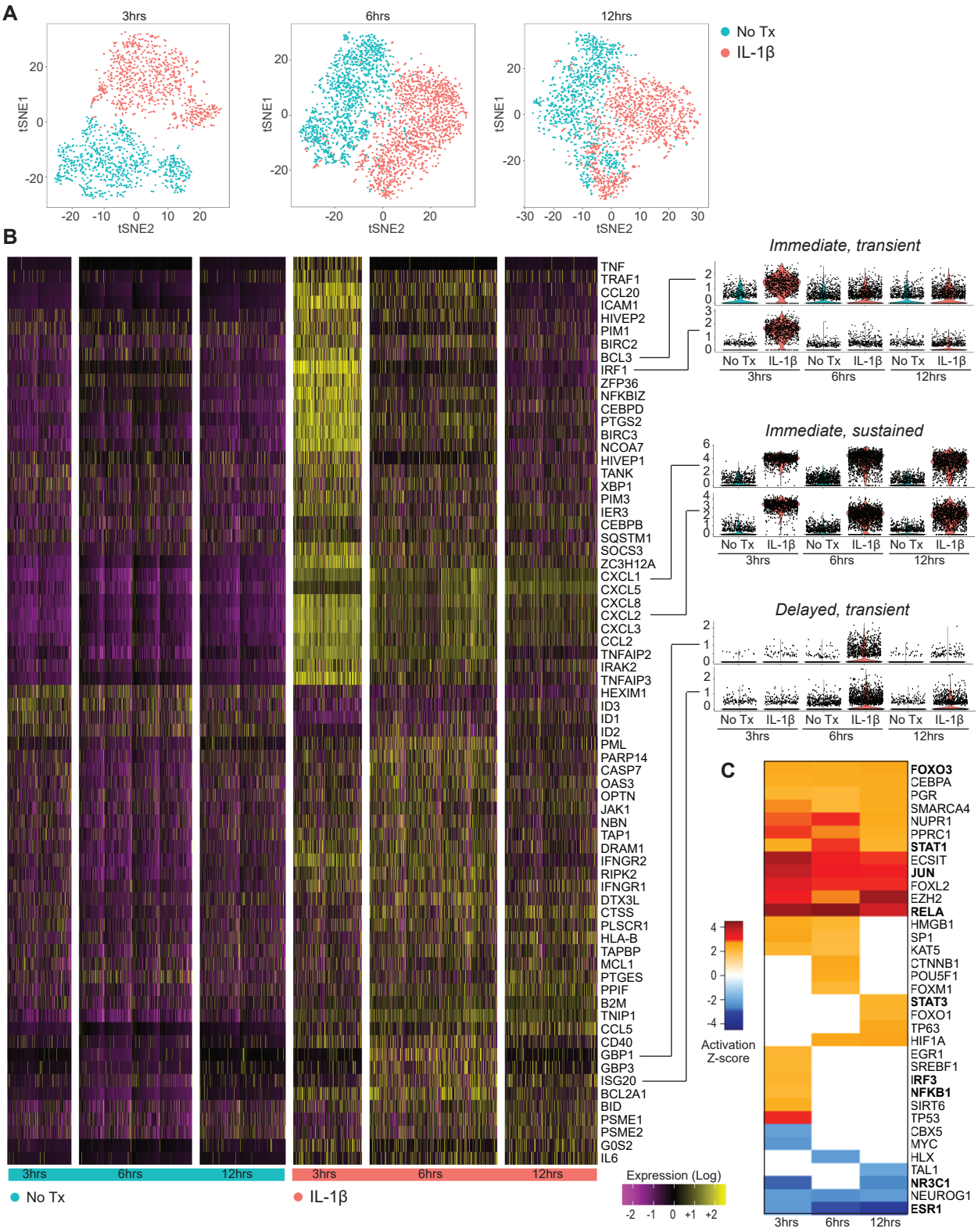


A. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IL-1R1-gRNA were treated with media (0) or 10ng/ml IL-1β for 12-36hrs before protein analysis by immunoblot

B. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IL-1R1-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-36hrs before qRT-PCR analysis. Statistical analysis was performed using student's T test with Holm-Sidak to compare genotypes, n=4 with mean ± SEM

**p<0.01, ***p<0.001

Figure S2. IL-1β initiates antiviral gene programs, Related to Figure 1

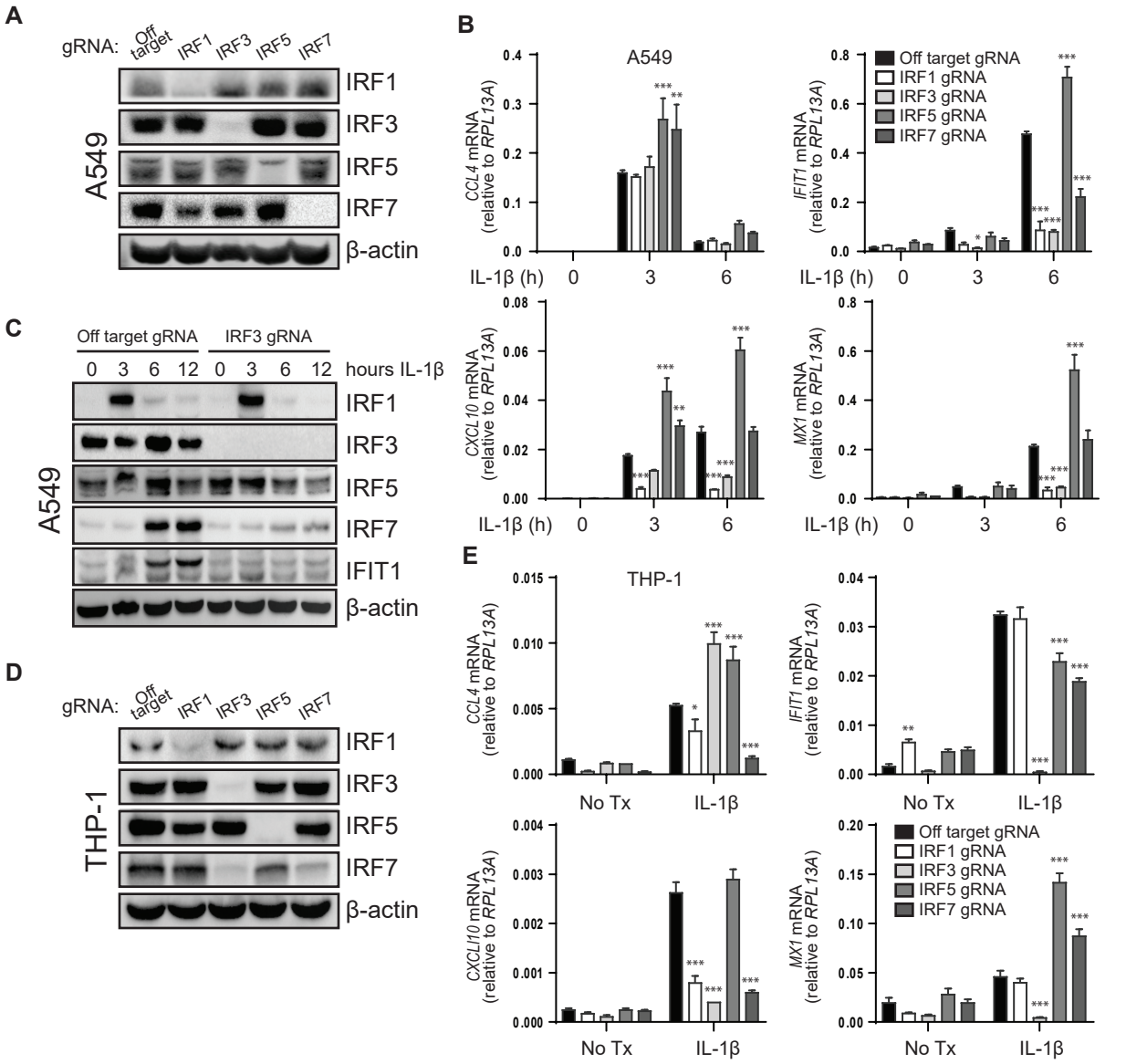


A. A549 were treated with media or 10ng/ml IL-1β for 3, 6 or 12hrs before single cells were isolated and prepared for sequencing. tSNE analysis of scRNA-seq expression with each point representing a single cell treated with media (blue) or IL-1β (orange)

B. Left: Gene expression heatmap of selected differentially expressed genes comparing media and IL-1β treatment. Right: Violin plots representing normalized gene expression of select genes per cell

C. Activation z-score heatmap of predicted transcription factor activity upon treatment with IL-1β

Figure S3. Differential IRF requirements for IL-1β-induced antiviral gene induction, Related to Figure 1



A. A549 were transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA before protein analysis by immunoblot

B. A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-6hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA and Dunnett's to compare knockouts to control cells, n=3 with mean ± SEM

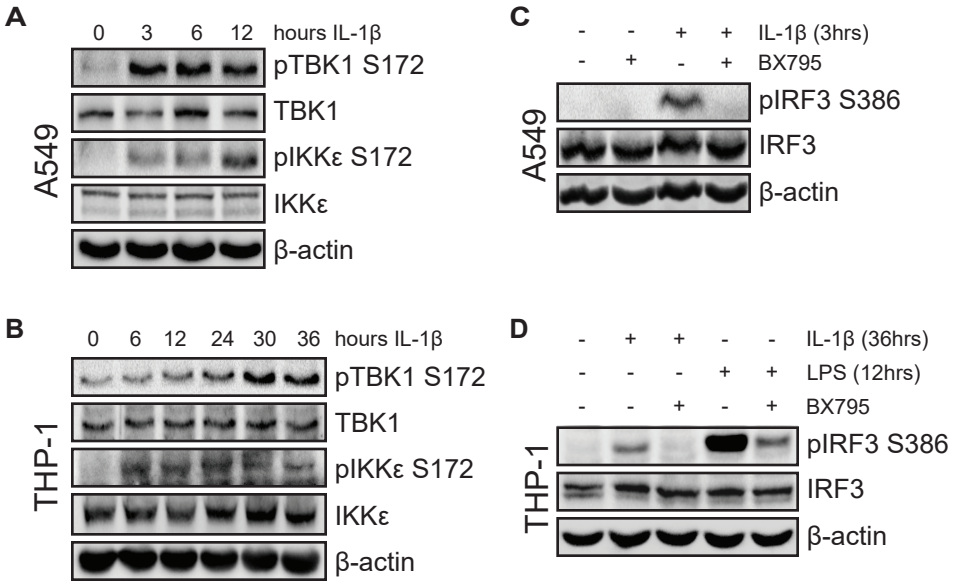
C. A549 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF3-gRNA were treated with media (0) or 10ng/ml IL-1β for 3-12hrs before protein analysis by immunoblot

D. THP-1 were transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA before protein analysis by immunoblot

E. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or IRF1-, IRF3-, IRF5- or IRF7-gRNA were treated with media (0) or 10ng/ml IL-1β for 36hrs before qRT-PCR analysis. Statistical analysis was performed using two-way ANOVA and Dunnett's to compare knockouts to control cells, n=4 with mean ± SEM

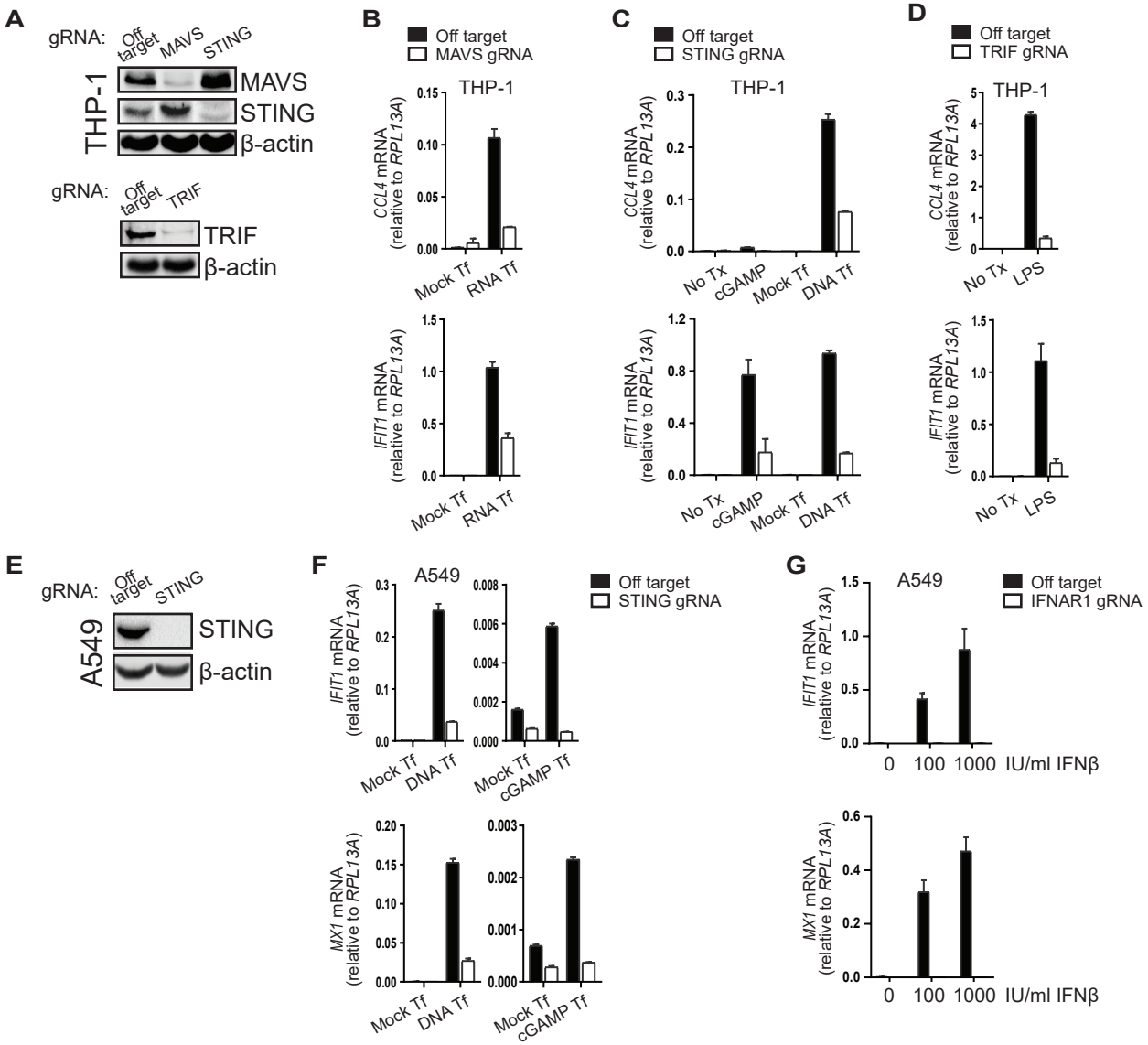
*p<0.05, **p<0.01, ***p<0.001

Figure S4. TBK1/IKKε mediate IL-1β-induced IRF3 phosphorylation, Related to Figure 2



A. A549 were treated with media (0) or 10ng/ml IL-1β for 3-12hrs before protein analysis by immunoblot
B. THP-1 were treated with media (0) or 10ng/ml IL-1β for 6-36hrs before protein analysis by immunoblot
C. A549 were treated with media (0) or 10ng/ml IL-1β for 3 hours +/- 1-hour pre-treatment with DMSO or 1uM BX795 before protein analysis by immunoblot
D. THP-1 were treated with media (0), 10ng/ml IL-1β for 36hrs or 0.5ug/ml LPS for 12hrs +/- 1hr pre-treatment with DMSO or 1uM BX795 before protein analysis by immunoblot

Figure S5. CRISPR-targeting analysis, Related to Figures 2 and 5



A. THP-1 were transduced with lentiCRISPR/Cas9 and off target gRNA or gRNA targeting MAVS, STING or TRIF before protein analysis by immunoblot

B. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or MAVS-gRNA were mock transfected or transfected with 0.1ug/ml PAMP RNA for 6hrs

C. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA were treated with media or 25ug/ml exogenous cGAMP, mock transfected or transfected with 1ug/ml calf thymus DNA for 6hrs

D. THP-1 transduced with lentiCRISPR/Cas9 and off target gRNA or TRIF-gRNA were treated with media or 0.5ug/ml LPS for 6hrs

E. A549 were transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA before protein analysis by immunoblot

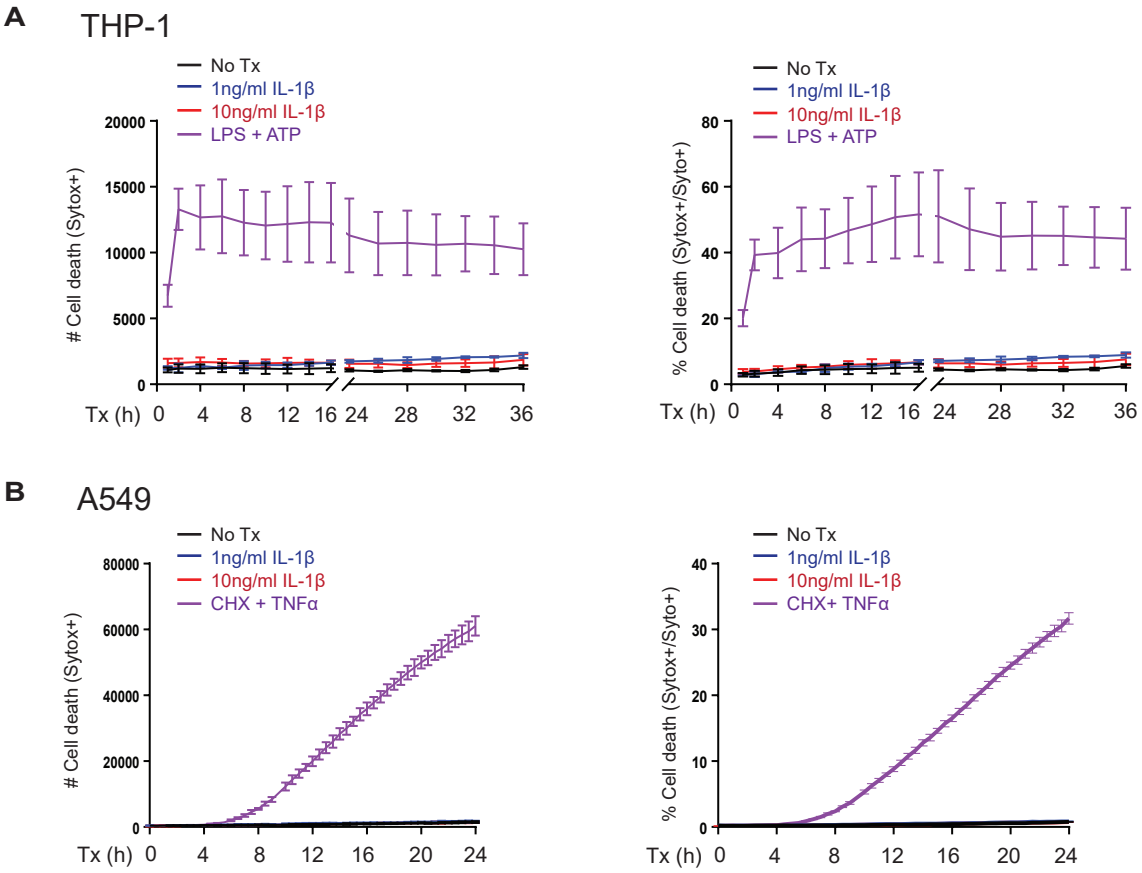
F. A549 transduced with lentiCRISPR/Cas9 and off target gRNA or STING-gRNA were mock transfected, transfected with 1ug/ml calf thymus DNA, or transfected with 10ug/ml cGAMP for 6hrs

G. A549 transfected with CRISPR/Cas9 and off target gRNA or IFNAR1-gRNA were treated with media or 100 or 1000IU/ml IFNβ for 12hrs.

B-D, F-G. After treatment, the indicated transcripts were analyzed by qRT-PCR; n=3 per genotype with mean ± SEM.

Tf, Transfection

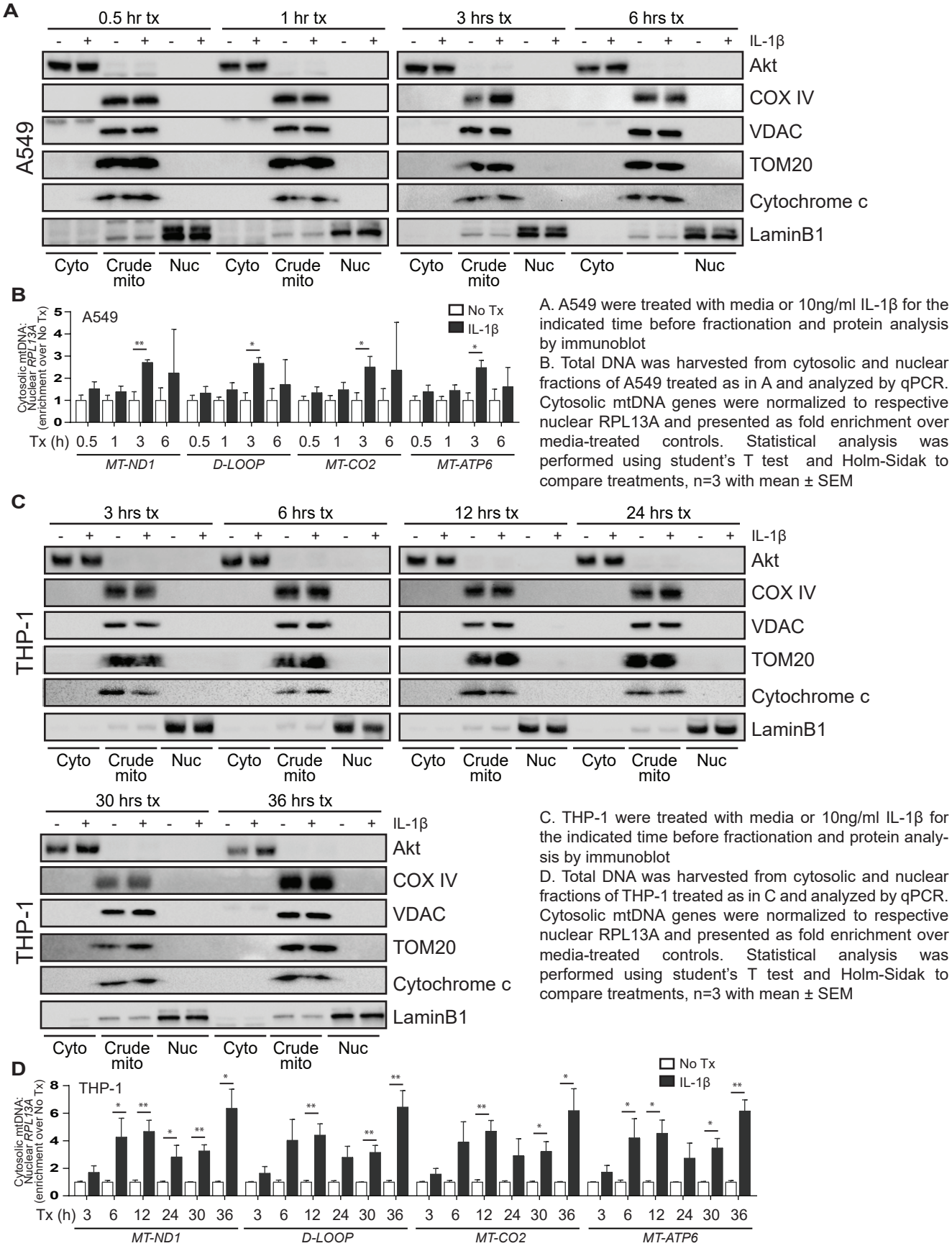
Figure S6. Exogenous IL-1β does not cause cell death, Related to Figure 3



A. THP-1 were treated with media or the indicated concentrations of IL-1β or 0.5ug/ml LPS + 5mM ATP and imaged by IncuCyte live cell imaging to quantify cell death over 36hrs. Left: Cell death number was quantified by counting Sytox-positive cells. Right: Percentage cell death was quantified by dividing Sytox-positive cells by Syto-positive cells. Data represent technical triplicates with mean ± SEM

B. A549 were treated with media or the indicated concentrations of IL-1β or 10ng/ml TNFα + 10uM Cycloheximide (CHX) and imaged by IncuCyte live cell imaging to quantify cell death over 24hrs. Left: Cell death number was quantified by counting Sytox-positive cells. Right: Percentage cell death was quantified by dividing Sytox-positive cells by Syto-positive cells. Data represent technical triplicates with mean ± SEM

Figure S7. Temporal release of mitochondrial DNA upon IL-1β treatment, Related to Figure 3



A. A549 were treated with media or 10ng/ml IL-1β for the indicated time before fractionation and protein analysis by immunoblot

B. Total DNA was harvested from cytosolic and nuclear fractions of A549 treated as in A and analyzed by qPCR. Cytosolic mtDNA genes were normalized to respective nuclear RPL13A and presented as fold enrichment over media-treated controls. Statistical analysis was performed using student's T test and Holm-Sidak to compare treatments, n=3 with mean ± SEM

C. THP-1 were treated with media or 10ng/ml IL-1β for the indicated time before fractionation and protein analysis by immunoblot

D. Total DNA was harvested from cytosolic and nuclear fractions of THP-1 treated as in C and analyzed by qPCR. Cytosolic mtDNA genes were normalized to respective nuclear RPL13A and presented as fold enrichment over media-treated controls. Statistical analysis was performed using student's T test and Holm-Sidak to compare treatments, n=3 with mean ± SEM

Table S2. Oligonucleotides, Related to Figures 3, 4 and 7

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Primer *MT-ND1* Fwd (CACCCAAGAACAGGGTTTGT)	(Aguirre, 2017)	N/A
Primer *MT-ND1* Rev (TGGCCATGGGTATGTTGTTAA)	(Aguirre, 2017)	N/A
Primer *D-LOOP* Fwd (CTATCACCCTATTAACCACTCA)	(Aguirre, 2017)	N/A
Primer *D-LOOP* Rev (TTCGCCTGTAATATTGAACGTA)	(Aguirre, 2017)	N/A
Primer *MT-CO2* Fwd (AATCGAGTAGTACTCCCGATTG)	(Aguirre, 2017)	N/A
Primer *MT-CO2* Rev (TTCTAGGACGATGGGCATGAAA)	(Aguirre, 2017)	N/A
Primer *MT-ATP6* Fwd (AATCCAAGCCTACGTTTTCACA)	(Aguirre, 2017)	N/A
Primer MT-ATP6 Rev (AGTATGAGGAGCGTTATGGAGT)	(Aguirre, 2017)	N/A
DENV-2 Fwd (TGTTGGTGCAACTCTACGTCCACA)	This paper	GenBank ID GQ398314.1
DENV-2 Rev (TGTGGAACGAGTGCCACtGATCTT)	This paper	GenBank ID GQ398314.1