nature microbiology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41564-017-0092-4

In the format provided by the authors and unedited.

AXL promotes Zika virus infection in astrocytes by antagonizing type I interferon signalling

Jian Chen[1], Yi-feng Yang[2], Yu Yang[1], Peng Zou[1], Jun Chen[3], Yongquan He[1], Sai-lan Shui[2], Yan-ru Cui[2], Ru Bai[1], Ya-jun Liang[2], Yunwen Hu[1], Biao Jiang[2], Lu Lu[1], Xiaoyan Zhang[1,4]*, Jia Liu[2]* and Jianqing Xu[1,4]*

[1]Scientific Research Center, Shanghai Public Health Clinical Center & Institutes of Biomedical Sciences, Key Laboratory of Medical Molecular Virology of Ministry of Education/Health, Shanghai Medical College, Fudan University, Shanghai, China. [2]Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai, China. [3]Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden. [4]State Key Laboratory for Infectious Disease Prevention and Control, China Centers for Disease Control and Prevention, Beijing, China. Jian Chen and Yi-feng Yang contributed equally to this work. Deceased: Yunwen Hu. *e-mail: zhangxiaoyan@shaphc.org.cn; liujia@shanghaitech.edu.cn; xujianqing@shaphc.org.cn

Supplementary Information

AXL Promotes Zika Virus Infection in Astrocytes by Antagonizing Type I Interferon Signaling

Jian Chen[1†], Yi-feng Yang[2†], Yu Yang[1], Peng Zou[1], Jun Chen[3], Yongquan He[1], Sai-lan Shui[2], Yan-ru Cui[2], Ru Bai[1], Ya-jun Liang[2], Yunwen Hu[1], Biao Jiang[2], Lu Lu[1], Xiaoyan Zhang[1,4*], Jia Liu[2*], Jianqing Xu[1,4*]

[1]Shanghai Public Health Clinical Center & Institutes of Biomedical Sciences, Key Laboratory of Medical Molecular Virology of Ministry of Education/Health, Shanghai Medical College, Fudan University, Shanghai, 201508, China

[2]Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai, 201210, China

[3]Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, 75236 Uppsala, Sweden

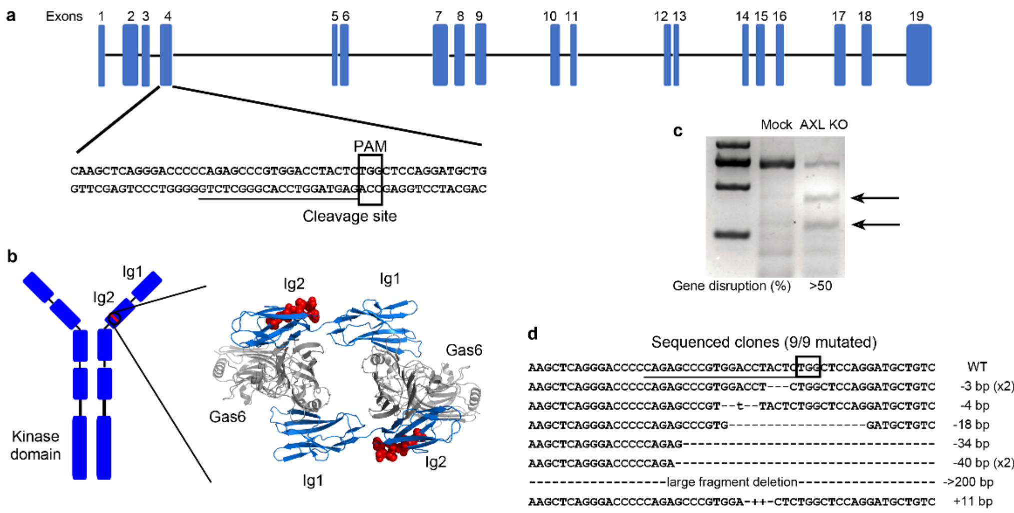
[4]State Key Laboratory for Infectious Disease Prevention and Control, China Centers for Disease Control and Prevention, Beijing, China

[5]The first two authors contributed equally to this work.

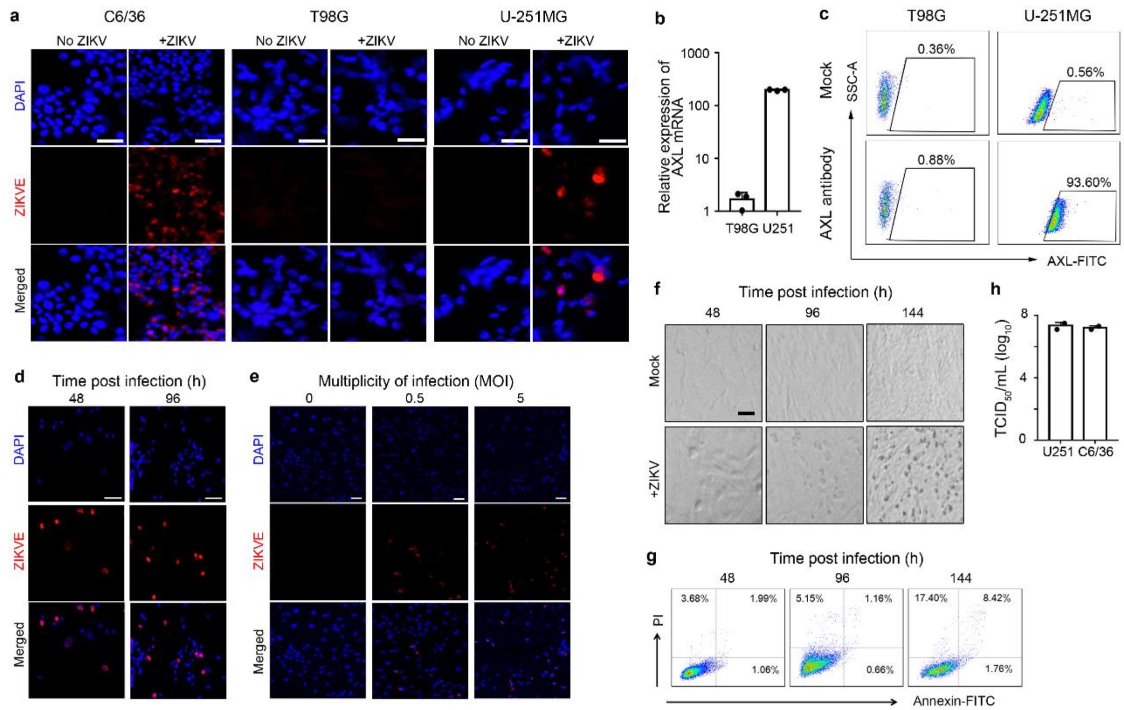
Correspondence should be addressed to: Jianqing Xu (xujianqing@shphc.org.cn), Jia Liu (liujia@shanghaitech.edu.cn) and Xiaoyan Zhang (zhangxiaoyan@shaphc.org.cn)

Table of Content

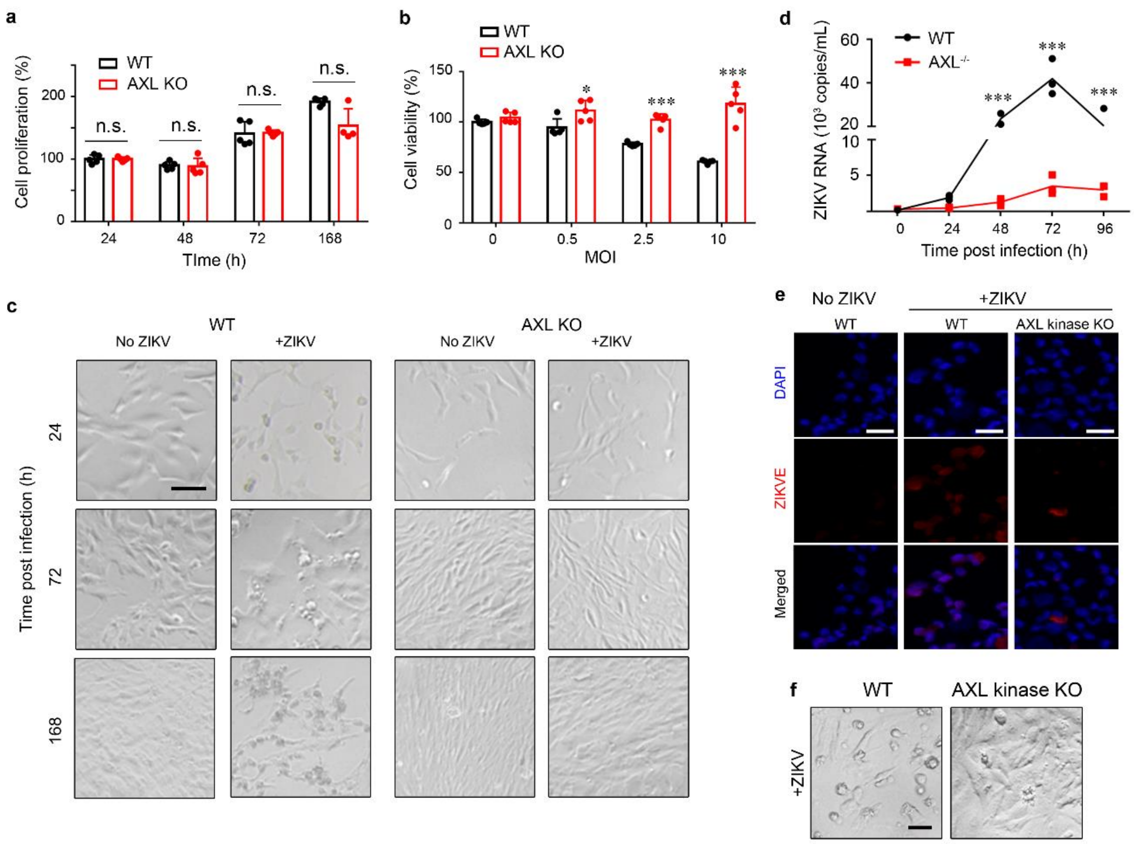
Supplementary Figures



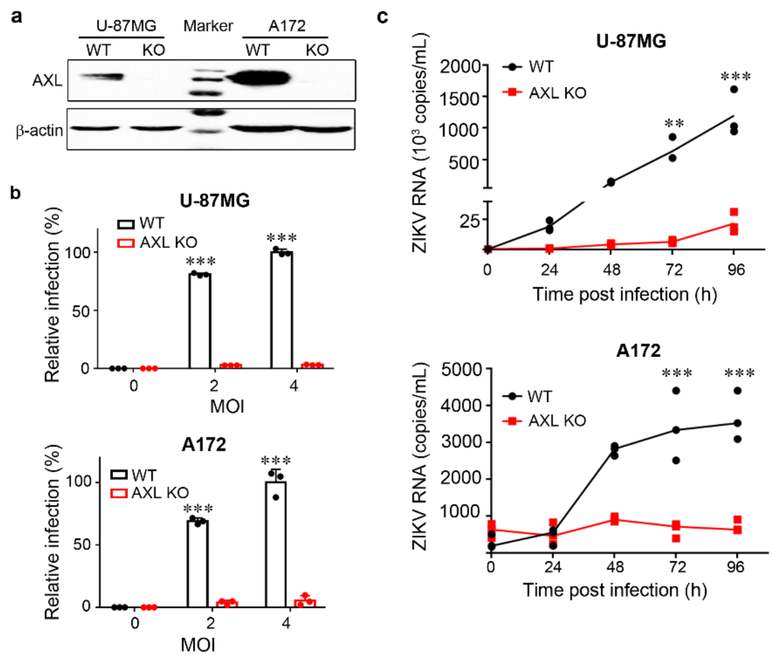
Supplementary Figure 1. Design and validation of CRISPR/Cas9 sgRNA targeting to the fourth exon of AXL. a, Design of sgRNA targeting to the fourth exon of AXL. **b,** Localization of sgRNA targeting site in AXL. Extracellular domain of AXL in complex with its ligand Gas6 is displayed using PyMOL (PDB entry 2C5D). **c** and **d,** Validation of designed sgRNA in U-251MG cells using T7E1 assay (**c**) and Sanger DNA sequencing (**d**). The arrows indicate cleavage products from T7E1 assay. The boxed and underlined nucleotides are PAM sequence and sgRNA targeting site, respectively.



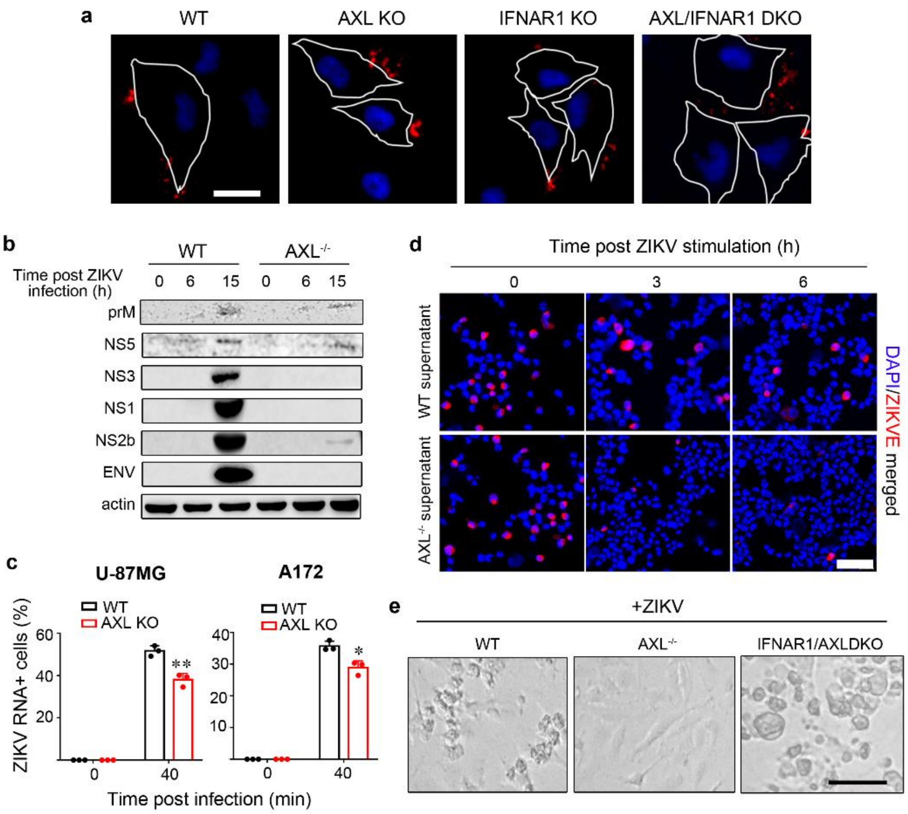
Supplementary Figure 2. Establishment of U-251MG as a model cell line for ZIKV infection. a, Identification of U-251MG astrocytoma as permissive cells for ZIKV infection. Scale bar, 20 μm. **b**, mRNA expression of AXL in T98G and U-251MG. The data in are from three biological replicates and shown as mean ± SD (n = 3). **c**, Surface expression of AXL on T98G and U-251MG cells. **d-e**, Time- (**d**) and dose (**e**)-dependent ZIKV infection in U-251MG. Scale bar, 50 μm. **f**, ZIKV-induced CPE in U-251MG. Scale bar, 100 μm. **g**, ZIKV-induced apoptosis of U-251MG cells, as determined by Annexin/propidium iodide (PI) stain. **h**, $TCID_{50}$ of ZIKV particles produced from U-251MG and C6/36 cells. The data in are from at least three biological replicates and shown as mean ± SD ($n \geq 3$). For **a**, **c**, **d**, **f** and **g**, the representative images from three independent experiments are shown.



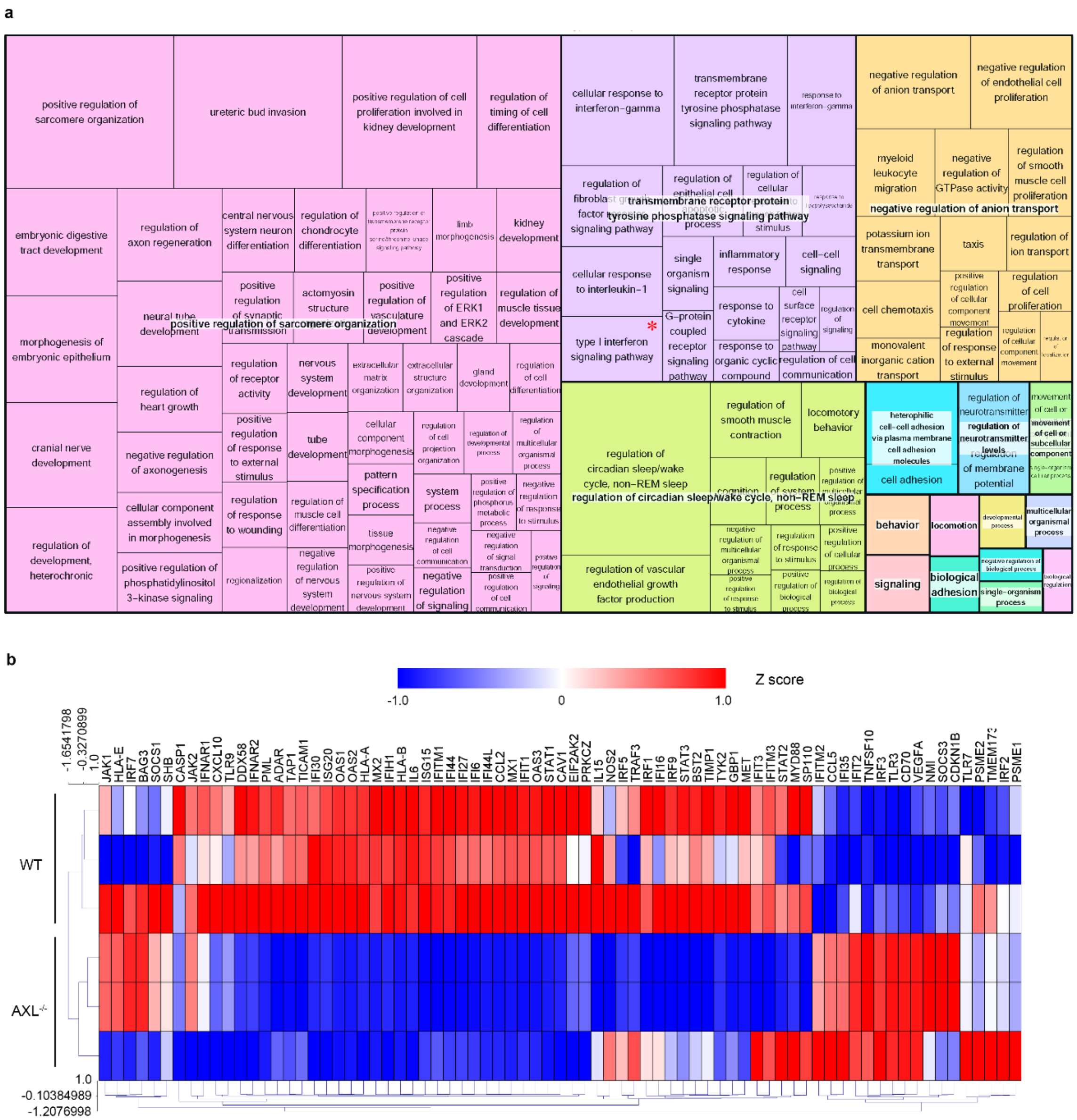
Supplementary Figure 3. AXL knockout in U-251MG prevents ZIKV infection and ZIKV-induced cell death. a, The effects of AXL KO on the proliferation of U-251MG cells. The data in are from five biological replicates and shown as mean ± SD (*n* = 5). Significant difference is determined by two-way ANOVA with Sidak's multiple comparison test (n.s., not significant). **b**, AXL KO prevents ZIKV-induced cell death at varying viral dose. The data in are from five biological replicates and shown as mean ± SD (*n* = 5). Significant difference is determined by two-way ANOVA with Sidak's multiple comparison test (*, $p = 0.023$; ***, $p < 0.0001$). **c**, AXL KO prevents ZIKV-induced CPEs. Scale bar, 100 μm. **d**, Viral replication in WT and AXL$^{-/-}$ U-251MG cells. The data in are from at least three biological replicates and shown as mean ± SD ($n \geq 3$). Significant difference between WT and AXL$^{-/-}$ cells at each time point is determined by two-way ANOVA (***, $p<0.0001$). **e-f**, Disruption of the kinase domain of AXL prevents ZIKV infection (**e**) and ZIKV-induced cell death (**f**). Scale bar, 100 μm. For **c**, **e** and **f**, The representative images from three independent experiments are shown.



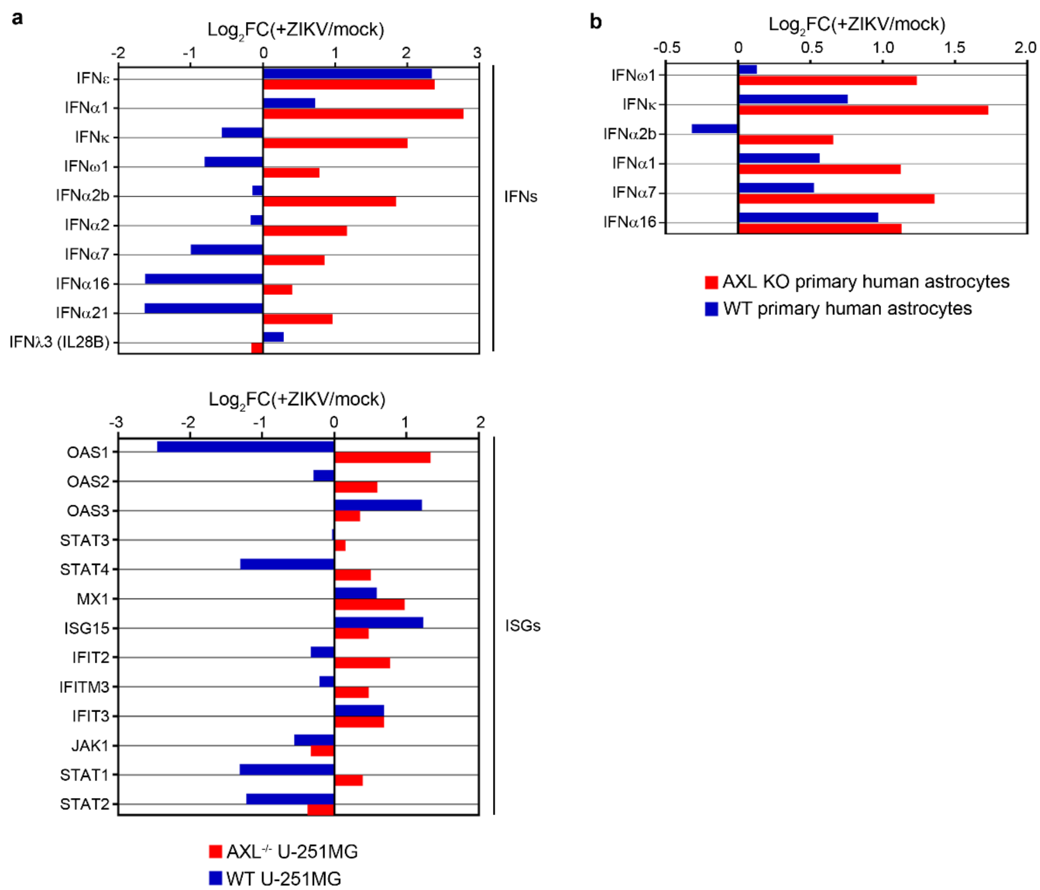
Supplementary Figure 4. AXL KO prevents ZIKV infection in U-87MG and A172 cells. a, Western blot analysis of AXL expression in WT and AXL KO U-87MG and A172 cells. The representative images from three independent experiments are shown. **b**, AXL KO prevents ZIKV infection in U-87MG and A172 cells, as determined by immunofluorescence. The results are from three biological replicates with 3,00 cells examined in each experiment. The data are shown as mean ± SD (n = 3). Significant difference between WT and AXL KO cells is determined by two-way ANOVA with Sidak's multiple comparison test (***, $p < 0.001$). **c**, AXL KO restricts ZIKV replication in U-87MG and A172 cells. The data are collected from at least three biologically independent experiments and are shown as mean ± SD ($n \geq 3$). Significant difference between WT and AXL KO cells is determined by two-way ANOVA with Sidak's multiple comparison test (**, $p = 0.0021$; ***, $p < 0.001$).



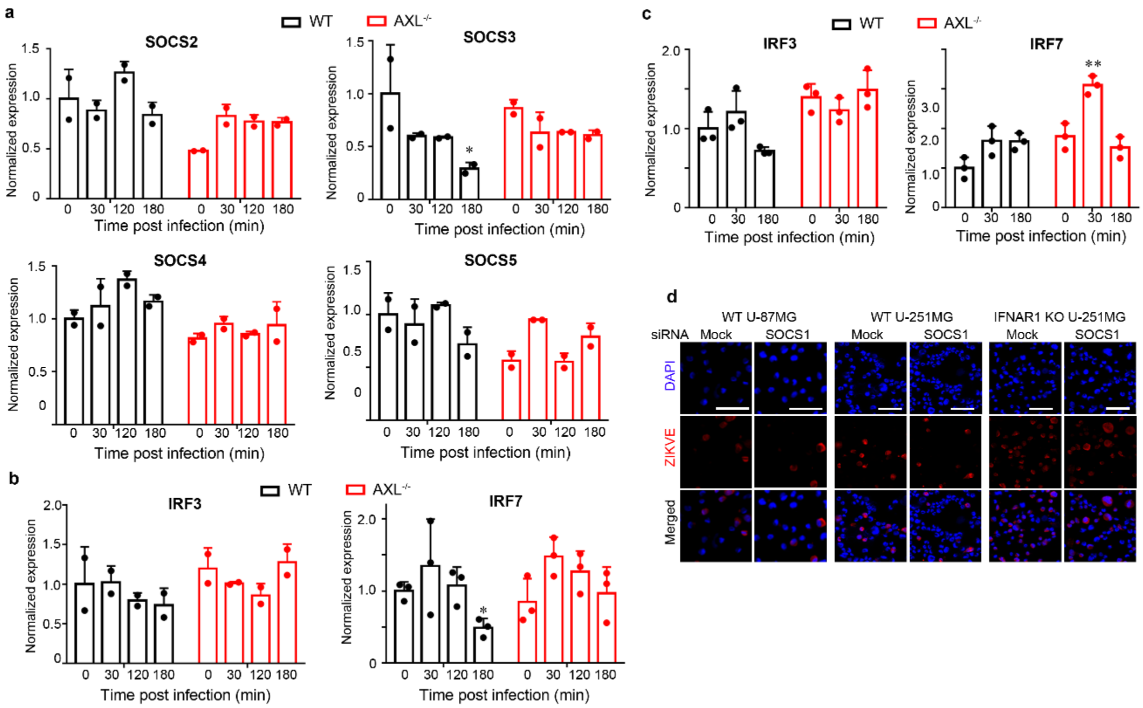
Supplementary Figure 5. AXL KO prevents ZIKV infection in astrocytes via IFNAR-dependent signaling. a, ZIKV entry assay under cold incubation conditions. Error bar, 20 μm. Membrane attached ZIKV was detected by anti-ZIKVE antibody. Scale bar, 10 μm. **b**, Production of ZIKV proteins in WT and AXL$^{-/-}$ U-251MG cells. **c**, ZIKV entry assay in WT and AXL KO U-87MG and A172 cells. The results are from three biological replicates with 3,00 cells examined in each experiment. The data are shown as mean ± SD (n = 3). Significant difference between WT and AXL KO cells is determined by Student's test (**, p = 0.0037; *, p = 0.011). **d**, Supernatant from ZIKV-stimulated AXL$^{-/-}$ U-251MG cells protects WT cells from ZIKV infection. Scale bar, 50 μm. **e**, IFNAR1 KO renders AXL$^{-/-}$ U-251MG cells susceptible to ZIKV-induced cell death. Scale bar, 100 μm. For **a**, **b**, **d** and **e**, the representative images from three independent experiments are shown.



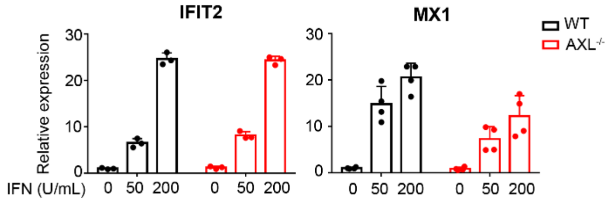
Supplementary Figure 6. RNA-Seq analyses of WT and AXL$^{-/-}$ U-251MG cells. a, REVIGO gene ontology (GO) tree map of down-regulated biological processes in AXL$^{-/-}$ cells compared with WT U-251MG cells. Significant GO terms (FDR≤0.05) with the same hierarchical branches (similarity ≥ 0.7) are grouped in the same colors. Significantly down-regulated type I IFN signaling pathway is donated with an asterisk. **b**, Z score of differentially expressed IFN responsive genes.



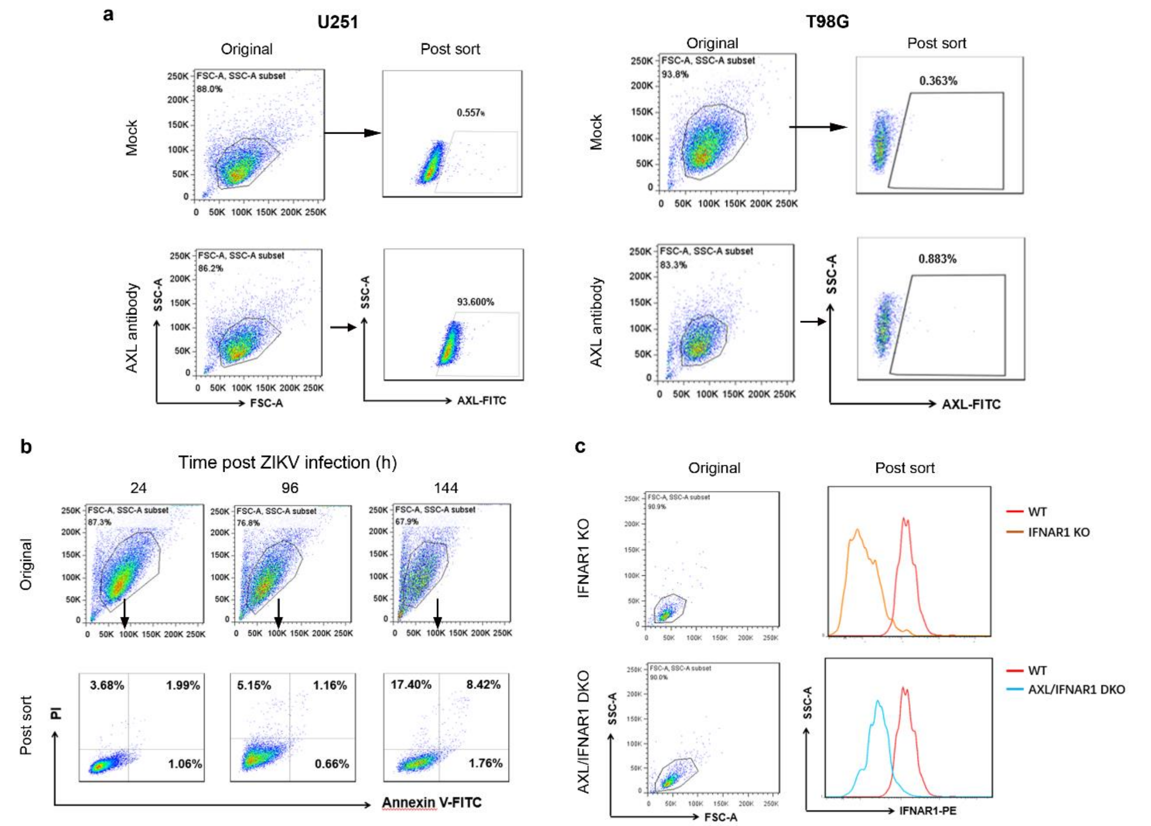
Supplementary Figure 7. RT-qPCR quantification of ZIKV-induced transcriptional change of IFNs and ISGs in U-251MG (a) and primary human astrocytes (b). Related to Figure 3a-b. Fold change of gene expression in response to ZIKV infection is presented.



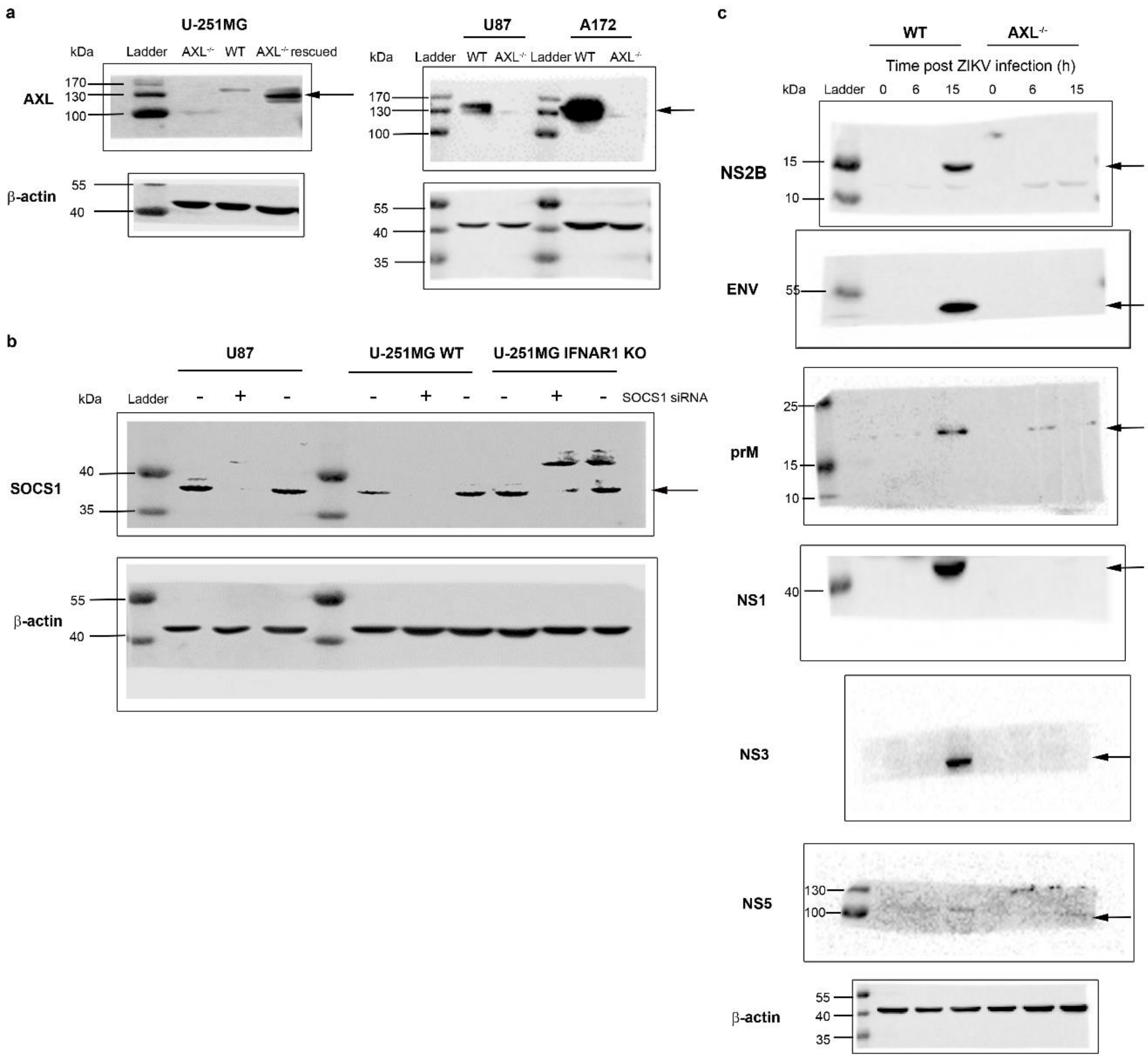
Supplementary Figure 8. Mechanism of AXL-mediated antagonism of type I IFN signaling during ZIKV infection. a, RT-qPCR quantification of the expression of SOCS proteins in U-251MG cells during ZIKV infection. The data are from two biological replicates and are shown as mean ± SD (n = 2). Significantly altered gene expression upon ZIKV infection is determined by two-way ANOVA with Sidak's multiple comparison test (*, p = 0.013). **b-c**, RT-qPCR quantification of IRF3 and IRF7 expression in U-251MG cells and primary astrocytes during ZIKV infection. The data are from three biological replicates and are shown as mean ± SD (n = 3). Significantly altered gene expression upon ZIKV infection is determined by two-way ANOVA with Sidak's multiple comparison test (*, p = 0.023; **, p = 0.0023). **d**, Immunofluorescence images of the effects of SOCS1 knock down on ZIKV infection in WT U-87MG, WT U-251MG and IFNAR1 KO U-251MG cells. Scale bar, 50 μm. The representative images from three independent experiments are shown.



Supplementary Figure 9. IFN-induced IFIT2 and MX1 expression in WT and AXL$^{-/-}$ U-251MG cells. The data are from four biological replicates and are shown as mean ± SD (n = 4).



Supplementary Figure 10. **Gating of flow cytometry samples. a**, AXL expression, related to Supplementary Fig. 2c. **b**, Annexin/PI stain, related to Supplementary Fig. 2g. **c**, expression of IFNAR1, related to Fig. 2d.



Supplementary Figure 11. Uncropped western blot images. **a**, AXL expression, related to Fig. 1f. **b**, SOCS1 expression, related to Fig. 3f. **c**, Expression of ZIKV proteins, related to Supplementary Fig. 5b. The arrows indicate protein of interest.

Supplementary Table

Supplementary Table 1. Primers list

Experiments	Primer name	Primer sequence
sgRNA synthesis	AXL-F	CACCGCAGAGCCCGTGGACCTACTC
sgRNA synthesis	AXL-R	AAACGAGTAGGTCCACGGGCTCTG
sgRNA synthesis	IFNAR1-F	CACCGGCTCGTCGCCGTGGCGCCAT
sgRNA synthesis	IFNAR1-R	AAACATGGCGCCACGGCGACGAGCC
T7E1	AXL Ext-FWD	GGGACATCAGACCTTCGTGT
T7E1	AXL Int-FWD	CAGAGGAAACCCAGGACAAA
T7E1	AXL Ext/Int-REV	TATTGGGGTCACGTGTGTGT
RT-qPCR	AXL-1F, 132 bp	CCGTGGACCTACTCTGGCT
RT-qPCR	AXL-1R, 132 bp	CCTTGGCGTTATGGGCTTC
RT-qPCR	SOCS1-F	GGTCCCCCTGGTTGTTGTA
RT-qPCR	SOCS1-R	TAGGAGGTGCGAGTTCAGGT
RT-qPCR	SOCS2-F	TTAAAAGAGGCACCAGAAGGAAC
RT-qPCR	SOCS2-R	AGTCGATCAGATGAACCACACT
RT-qPCR	SOCS3-F	CCTGCGCCTCAAGACCTTC
RT-qPCR	SOCS3-R	GTCACTGCGCTCCAGTAGAA
RT-qPCR	SOCS4-F	GCCGACAGAAAAGACGGTTAT
RT-qPCR	SOCS4-R	GGTTCCTTAAAGACACTTCGGTT
RT-qPCR	SOCS5-F	AGAGATTCCTACTCTCGACATGC
RT-qPCR	SOCS5-R	GCCCACAGTATCCTGCAACC
RT-qPCR	IRF3-F	AGAGGCTCGTGATGGTCAAG
RT-qPCR	IRF3-R	AGGTCCACAGTATTCTCCAGG
RT-qPCR	IRF7-F	GCTGGACGTGACCATCATGTA
RT-qPCR	IRF7-R	GGGCCGTATAGGAACGTGC
RT-qPCR	GAPDH-F	GCCTCCTGCACCACCAACTG
RT-qPCR	GAPDH-R	ACGCCTGCTTCACCACCTTC
RT-qPCR	IFITM3-F	GTCCACCGTGATCCACATCC
RT-qPCR	IFITM3-R	TCGCCAACCATCTTCCTGT
RT-qPCR	ISG15-F	CGCAGATCACCCAGAAGATT
RT-qPCR	ISG15-R	GCCCTTGTTATTCCTCACCA
RT-qPCR	MX1-F	GCAAATCAAGGCACTGGAAG
RT-qPCR	MX1-R	TCGAAACATCTGTGAAAGCAAG
RT-qPCR	OAS1-F	TGTCCAAGGTGGTAAAGGGTG
RT-qPCR	OAS1-R	CCGGCGATTTAACTGATCCTG
RT-qPCR	OAS3-F	GATGTGCTGCCAGCCTTTGA
RT-qPCR	OAS3-R	TGTAGCTCTGTGAAGCAGGTGGA
RT-qPCR	STAT1-F	ATCAGGCTCAGTCGGGGAATA

RT-qPCR	STAT1-R	TGGTCTCGTGTTCTCTGTTCT
RT-qPCR	STAT2-F	CCAGCTTTACTCGCACAGC
RT-qPCR	STAT2-R	AGCCTTGGAATCATCACTCCC
RT-qPCR	JAK1-F	CTTTGCCCTGTATGACGAGAAC
RT-qPCR	JAK1-R	ACCTCATCCGGTAGTGGAGC
RT-qPCR	IFIT2-F	GACACGGTTAAAGTGTGGAGG
RT-qPCR	IFIT2-R	TCCAGACGGTAGCTTGCTATT
RT-qPCR	IFIT3-F	AAAAGCCCAACAACCCAGAAT
RT-qPCR	IFIT3-R	CGTATTGGTTATCAGGACTCAGC
RT-qPCR	STAT3-F	ACCAGCAGTATAGCCGCTTC
RT-qPCR	STAT3-R	GCCACAATCCGGGCAATCT
RT-qPCR	STAT4-F	TGTTGGCCCAATGGATTGAAA
RT-qPCR	STAT4-R	GGAAACACGACCTAACTGTTCAT
RT-qPCR	IFNβ-F	GAGCTACAACTTGCTTGGATTC
RT-qPCR	IFNβ-R	CAAGCCTCCCATTCAATTGC
RT-qPCR	IFNE-F	GGCCTCTACCACTATCTTCTCTC
RT-qPCR	IFNE-R	ACACTGCTGAATTGACAAGGTTT
RT-qPCR	IFNW1-F	GAAGGCCCATGTCATGTCTGT
RT-qPCR	IFNW1-R	GAGTTGGTCTAGGAGGGTCAT
RT-qPCR	IFNK-F	GTGGCTTGAGATCCTTATGGGT
RT-qPCR	IFNK-R	CAGATTTTGCCAGGTGACTCTT
RT-qPCR	IL28B (IFNL3)-F	TAAGAGGGCCAAAGATGCCTT
RT-qPCR	IL28B (IFNL3)-R	CTGGTCCAAGACATCCCCC
RT-qPCR	IFNA1-F	GCCTCGCCCTTTGCTTTACT
RT-qPCR	IFNA1-R	CTGTGGGTCTCAGGGAGATCA
RT-qPCR	IFNA 2b-F	GCTTGGGATGAGACCCTCCTA
RT-qPCR	IFNA 2b-R	CCCACCCCCTGTATCACAC
RT-qPCR	IFNA2- F	GCTTGGGATGAGACCCTCCTA
RT-qPCR	IFNA2-R	CCCACCCCCTGTATCACAC
RT-qPCR	IFNA7-F	AGGGCCTTGATACTCCTGG
RT-qPCR	IFNA7-R	TCCTCCTCCGGGAATCTGAAT
RT-qPCR	IFNA21-F	AGGGCCTTGATACTCCTGG
RT-qPCR	IFNA21-R	TCTTGGGGGAATCCAAAGTCA
RT-qPCR	IFNA16-F	GGAGGGCCTTGATACTCCTG
RT-qPCR	IFNA16-R	CCTGGGGGAATCCGAAATCAT
RT-qPCR	IFNA7-F	AGGGCCTTGATACTCCTGG
RT-qPCR	IFNA7-R	TCCTCCTCCGGGAATCTGAAT

Supplementary Notes

AXL open reading frame (ORF)

>ATGGCGTGGCGGTGCCCCAGGATGGGCAGGGTCCCGCTGGCCTGGTGCTTGGCGCTGTGCGGCTGGGCGTGCATGGCCCCCAGGGGCAC
GCAGGCTGAAGAAAGTCCCTTCGTGGGCAACCCAGGGAATATCACAGGTGCCCGGGGACTCACGGGCACCCTTCGGTGTCAGCTCCAGGT
TCAGGGAGAGCCCCCCGAGGTACATTGGCTTCGGGATGGACAGATCCTGGAGCTCGCGGACAGCACCCAGACCCAGGTGCCCCTGGGTGA
GGATGAACAGGATGACTGGATAGTGGTCAGCCAGCTCAGAATCACCTCCCTGCAGCTTTCCGACACGGGACAGTACCAGTGTTTGGTGTT
TCTGGGACATCAGACCTTCGTGTCCCAGCCTGGCTATGTTGGGCTGGAGGGCTTGCCTTACTTCCTGGAGGAGCCCGAAGACAGGACTGT
GGCCGCCAACACCCCCTTCAACCTGAGCTGCCAAGCTCAGGGACCCCCAGAGCCCGTGGACCTACTCTGGCTCCAGGATGCTGTCCCCCT
GGCCACGGCTCCAGGTCACGGCCCCCAGCGCAGCCTGCATGTTCCAGGGCTGAACAAGACATCCTCTTTCTCCTGCGAAGCCCATAACGC
CAAGGGGGTCACCACATCCCGCACAGCCACCATCACAGTGCTCCCCCAGCAGCCCCGTAACCTCCACCTGGTCTCCCGCCAACCCACGGA
GCTGGAGGTGGCTTGGACTCCAGGCCTGAGCGGCATCTACCCCCTGACCCACTGCACCCTGCAGGCTGTGCTGTCAGACGATGGGATGGG
CATCCAGGCGGGAGAACCAGACCCCCCAGAGGAGCCCCTCACCTCGCAAGCATCCGTGCCCCCCCATCAGCTTCGGCTAGGCAGCCTCCA
TCCTCACACCCCTTATCACATCCGCGTGGCATGCACCAGCAGCCAGGGCCCCTCATCCTGGACCCACTGGCTTCCTGTGGAGACGCCGGA
GGGAGTGCCCCTGGGCCCCCCTGAGAACATTAGTGCTACGCGGAATGGGAGCCAGGCCTTCGTGCATTGGCAAGAGCCCCGGGCGCCCCT
GCAGGGTACCCTGTTAGGGTACCGGCTGGCGTATCAAGGCCAGGACACCCCAGAGGTGCTAATGGACATAGGGCTAAGGCAAGAGGTGAC
CCTGGAGCTGCAGGGGGACGGGTCTGTGTCCAATCTGACAGTGTGTGTGGCAGCCTACACTGCTGCTGGGGATGGACCCTGGAGCCTCCC
AGTACCCCTGGAGGCCTGGCGCCCAGGGCAAGCACAGCCAGTCCACCAGCTGGTGAAGGAACCTTCAACTCCTGCCTTCTCGTGGCCCTG
GTGGTATGTACTGCTAGGAGCAGTCGTGGCCGCTGCCTGTGTCCTCATCTTGGCTCTCTTCCTTGTCCACCGGCGAAAGAAGGAGACCCG
TTATGGAGAAGTGTTTGAACCAACAGTGGAAAGAGGTGAACTGGTAGTCAGGTACCGCGTGCGCAAGTCCTACAGTCGTCGGACCACTGA
AGCTACCTTGAACAGCCTGGGCATCAGTGAAGAGCTGAAGGAGAAGCTGCGGGATGTGATGGTGGACCGGCACAAGGTGGCCCTGGGGAA
GACTCTGGGAGAGGGAGAGTTTGGAGCTGTGATGGAAGGCCAGCTCAACCAGGACGACTCCATCCTCAAGGTGGCTGTGAAGACGATGAA
GATTGCCATCTGCACGAGGTCAGAGCTGGAGGATTTCCTGAGTGAAGCGGTCTGCATGAAGGAATTTGACCATCCCAACGTCATGAGGCT
CATCGGTGTCTGTTTCCAGGGTTCTGAACGAGAGAGCTTCCCAGCACCTGTGGTCATCTTACCTTTCATGAAACATGGAGACCTACACAG
CTTCCTCCTCTATTCCCGGCTCGGGGACCAGCCAGTGTACCTGCCCACTCAGATGCTAGTGAAGTTCATGGCAGACATCGCCAGTGGCAT
GGAGTATCTGAGTACCAAGAGATTCATACACCGGGACCTGGCGGCCAGGAACTGCATGCTGAATGAGAACATGTCCGTGTGTGTGGCGGA
CTTCGGGCTCTCCAAGAAGATCTACAATGGGGACTACTACCGCCAGGGACGTATCGCCAAGATGCCAGTCAAGTGGATTGCCATTGAGAG
TCTAGCTGACCGTGTCTACACCAGCAAGAGCGATGTGTGGTCCTTCGGGGTGACAATGTGGGAGATTGCCACAAGAGGCCAAACCCCATA
TCCGGGCGTGGAGAACAGCGAGATTTATGACTATCTGCGCCAGGGAAATCGCCTGAAGCAGCCTGCGGACTGTCTGGATGGACTGTATGC
CTTGATGTCGCGGTGCTGGGAGCTAAATCCCCAGGACCGGCCAAGTTTTACAGAGCTGCGGGAAGATTTGGAGAACACACTGAAGGCCTT
GCCTCCTGCCCAGGAGCCTGACGAAATCCTCTATGTCAACATGGATGAGGGTGGAGGTTATCCTGAACCCCCTGGAGCTGCAGGAGGAGC
TGACCCCCCAACCCAGCCAGACCCTAAGGATTCCTGTAGCTGCCTCACTGCGGCTGAGGTCCATCCTGCTGGACGCTATGTCCTCTGCCC
TTCCACAACCCCTAGCCCCGCTCAGCCTGCTGATAGGGGCTCCCCAGCAGCCCCAGGGCAGGAGGATGGTGCCTGA

IFNAR1 ORF

>ATGATGGTCGTCCTCCTGGGCGCGACGACCCTAGTGCTCGTCGCCGTGGCGCCATGGGTGTTGTCCGCAGCCGCAGGTGGAAAAAATC
TAAAATCTCCTCAAAAAGTAGAGGTCGACATCATAGATGACAACTTTATCCTGAGGTGGAACAGGAGCGATGAGTCTGTCGGGAATGTGA
CTTTTTCATTCGATTATCAAAAAACTGGGATGGATAATTGGATAAAATTGTCTGGGTGTCAGAATATTACTAGTACCAAATGCAACTTTT

CTTCACTCAAGCTGAATGTTTATGAAGAAATTAAATTGCGTATAAGAGCAGAAAAAGAAAACACTTCTTCATGGTATGAGGTTGACTCAT

TTACACCATTTCGCAAAGCTCAGATTGGTCCTCCAGAAGTACATTTAGAAGCTGAAGATAAGGCAATAGTGATACACATCTCTCCTGGAA

CAAAAGATAGTGTTATGTGGGCTTTGGATGGTTTAAGCTTTACATATAGCTTAGTTATCTGGAAAAACTCTTCAGGTGTAGAAGAAAGGA

TTGAAAATATTTATTCCAGACATAAAATTTATAAACTCTCACCAGAGACTACTTATTGTCTAAAAGTTAAAGCAGCACTACTTACGTCAT

GGAAAATTGGTGTCTATAGTCCAGTACATTGTATAAAGACCACAGTTGAAAATGAACTACCTCCACCAGAAAATATAGAAGTCAGTGTCC

AAAATCAGAACTATGTTCTTAAATGGGATTATACATATGCAAACATGACCTTTCAAGTTCAGTGGCTCCACGCCTTTTTAAAAAGGAATC

CTGGAAACCATTTGTATAAATGGAAACAAATACCTGACTGTGAAAATGTCAAAACTACCCAGTGTGTCTTTCCTCAAAACGTTTTCCAAA

AAGGAATTTACCTTCTCCGCGTACAAGCATCTGATGGAAATAACACATCTTTTTGGTCTGAAGAGATAAAGTTTGATACTGAAATACAAG

CTTTCCTACTTCCTCCAGTCTTTAACATTAGATCCCTTAGTGATTCATTCCATATCTATATCGGTGCTCCAAAACAGTCTGGAAACACGC

CTGTGATCCAGGATTATCCACTGATTTATGAAATTATTTTTTGGGAAAACACTTCAAATGCTGAGAGAAAAATTATCGAGAAAAAAACTG

ATGTTACAGTTCCTAATTTGAAACCACTGACTGTATATTGTGTGAAAGCCAGAGCACACACCATGGATGAAAAGCTGAATAAAAGCAGTG

TTTTTAGTGACGCTGTATGTGAGAAAACAAAACCAGGAAATACCTCTAAAATTTGGCTTATAGTTGGAATTTGTATTGCATTATTTGCTC

TCCCGTTTGTCATTTATGCTGCGAAAGTCTTCTTGAGATGCATCAATTATGTCTTCTTTCCATCACTTAAACCTTCTTCCAGTATAGATG

AGTATTTCTCTGAACAGCCATTGAAGAATCTTCTGCTTTCAACTTCTGAGGAACAAATCGAAAAATGTTTCATAATTGAAAATATAAGCA

CAATTGCTACAGTAGAAGAAACTAATCAAACTGATGAAGATCATAAAAAATACAGTTCCCAAACTAGCCAAGATTCAGGAAATTATTCTA

ATGAAGATGAAAGCGAAAGTAAAACAAGTGAAGAACTACAGCAGGACTTTGTATGA