SUPPLEMENTARY INFORMATION

Trex1 regulates lysosomal biogenesis and interferon-independent activation of antiviral genes

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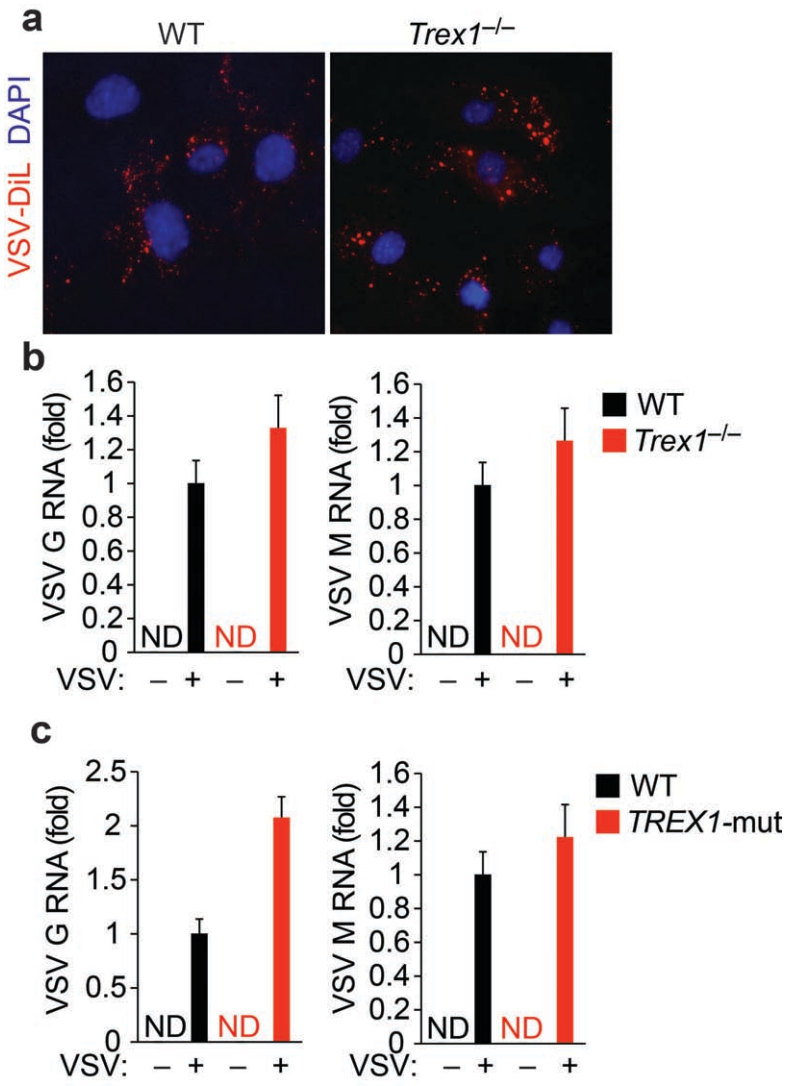
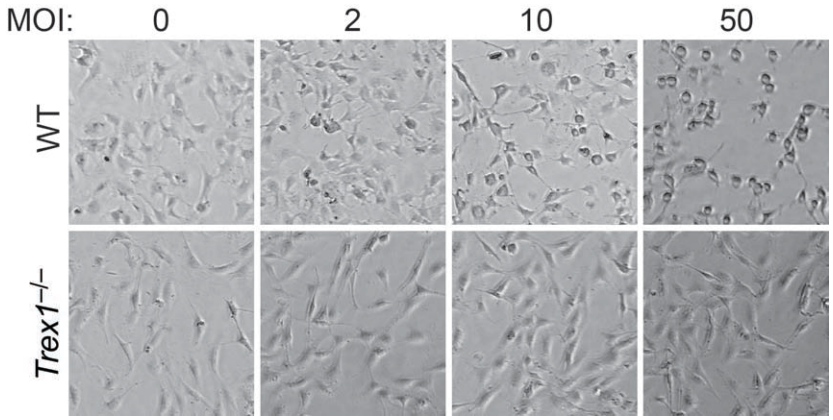
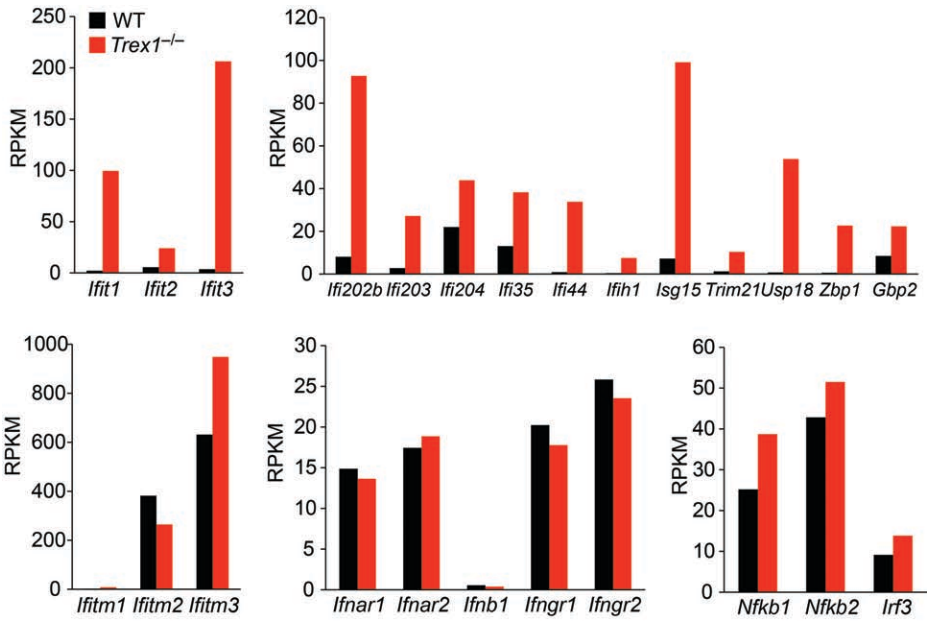


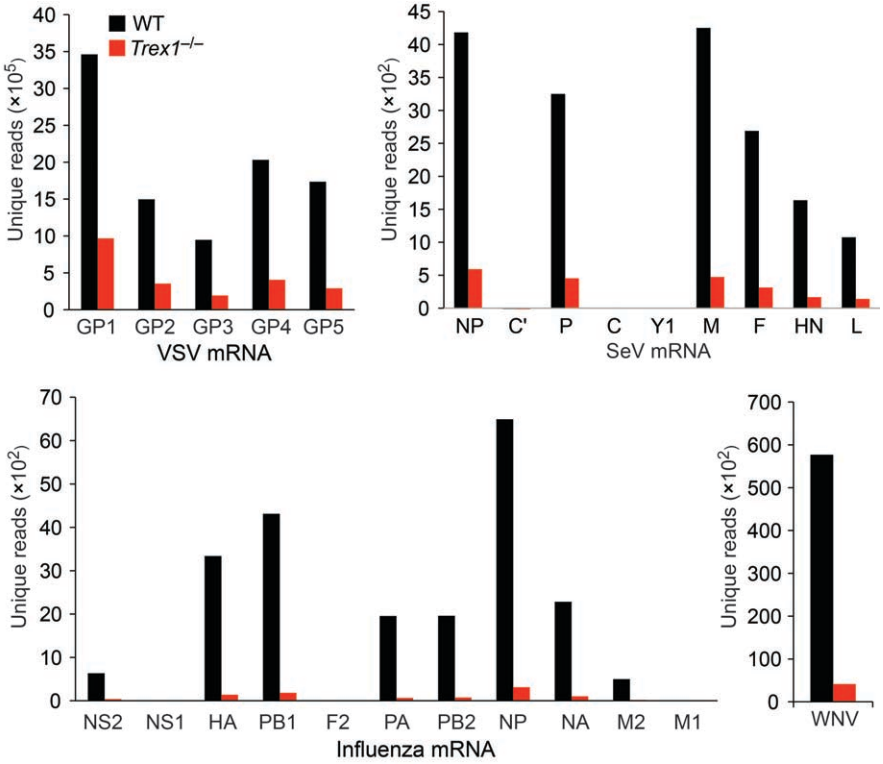
Supplementary Figure 1. VSV entry is not affected by Trex1-deficiency or *TREX1* mutation. (**a**) Fluorescent microscopy of WT and *Trex1*$^{-/-}$ MEFs infected with VSV-DiL (red, fluorescently labeled virion) for 1 h. Representative images from 3 independent experiments are shown. (**b,c**) Quantitative RT-PCR analysis of VSV G and M RNA in WT and *Trex1*$^{-/-}$ MEFs (**b**) or WT and *TREX1*$^{R114H/R114H}$ (*TREX1*-mut) human fibroblasts (**c**) infected with VSV at MOI of 2 for 1 h. WT normalized to 1. ND, not detectable. Data are representative of two independent experiments (error bars, s.d.).



Supplementary Figure 2. VSV infection caused cytopathic effect in WT, but not *Trex1*$^{-/-}$, MEFs. WT and *Trex1*$^{-/-}$ MEFs were infected with VSV at MOI indicated on top for 18 h. Phase contrast images were taken without fixing the cells. Representative images from three independent experiments are shown.



Supplementary Figure 3. Selected host gene expression from RNA-SEQ analysis in Fig. 3a. RPKM, Reads Per Kilobase of exon model per Million mapped reads, indicates expression value of each gene.

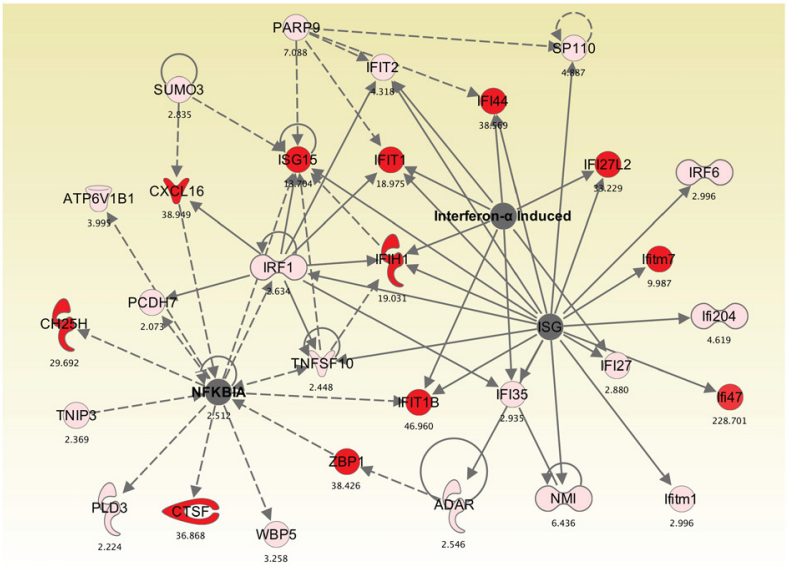


Supplementary Figure 4. Selected viral gene expression from RNA-SEQ analysis in Fig. 3a. Viral mRNAs that are not polyadenylated or do not contain A rich sequences will not be detected by RNA-SEQ, due to a preparation step that involves poly-dT selection.

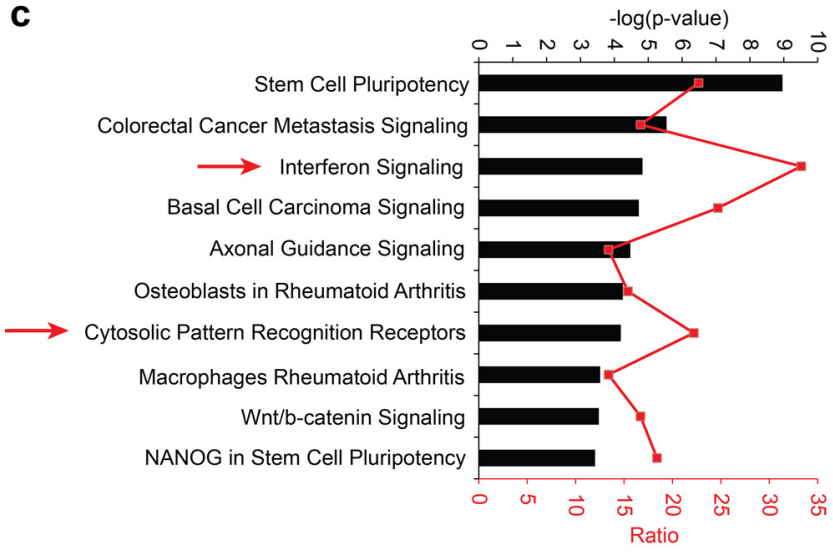
a

Rank	Network function	Score	Molecules in network
1	Antimicrobial Response, Inflammatory Response, Infectious Disease	40	ADAR,ATP6V1B1,CH25H,CTSF,CXCL16,Ifitm1,GBP2,IFI27,IFI35,IFI44,Ifi47,Ifi204,IFI27L2,IFIH1,IFIT1,IFIT2,IFIT1B,Ifitm7,IRF6,IRF1,IRG,ISG15,NFKBIA,NMI,PARP9,PCDH7,PLD3,RNASE4,SP110,SUMO3,TNFSF10,TNIP3,WBP5,ZBP1
2	Genetic Disorder, Neurological Disease, Psychological Disorders	38	ADRA1D,BAI2,CD97,CELSR2,CYSLTR1,DRD4,F2RL3,FZD1,FZD3,FZD6,FZD7,FZD9,Gpcr,GPR44,GPR56,GPR61,GPR75,GPR88,GPR97,GPR115,GPR124,GPR132,GPR133,GPR157,GPR162,GPR176,GPR137B,GPRC5C,HRH2,HTR6,KISS1R,NPY1R,OPRL1,Pik3r
3	Cellular Movement, Connective Tissue Development and Function, Cellular Function and Maintenance	36	AASS,AChR,Actg2,AGRN,C13orf15,,CPXM1,DFNA5,DNAH2,Ecm,ECM2,GALM,GDPD5,GYLTL1B,H1FX,LOXL2,Masp1,MFAP2,NOV,OVOL1,PDK2,PDZK1IP1,PHKG1,phosphorylase,PLA1A,RAB9A,RAMP2,ROBO3,SERPINB10,SPAG4,SPARCL1,STARD10,TGFB3,TGFB1,TYMP
4	Amino Acid Metabolism, Energy Production, Post-Translational Modification	36	AIFM3,ARNT2,BIRC3,CASQ1,CD53,FAM43A,IFI44L,Ifng,IKK,IMPACT,L1CAM,MALT1,MDM4,NLRP2,NPAS1,PEA15,PEG3,PKP3,PSEN2,RALGPS2,RHPN2,Rpl29,RPS6KA1,Serpina3k,SFN,SGK223,SHROOM1,SOX7,Sp100,ST5,Tnf,UACA,XAF1,Zfp108/Zfp93
5	Drug Metabolism, Lipid Metabolism, Small Molecule Biochemistry	34	ARSG,ARSI,ARSK,Cml5,CYP1A1,Cyp1a,CYP27A1,CYP2C18,CYP2D6,CYP2F1,Cyp2j9,CYP3A4,CYP7B1,FMO5,GAB1,GTPASE,NAALAD2,PRPH,PTPLAD1,RASA4/RASA4B,RBFOX1,RGS16,RGS17,RORA,Shank2,SIDT2,SLC46A3,ST3GAL4,TSC22D1,UGT,UGT1A6,Ugt1a7c,VEPH1

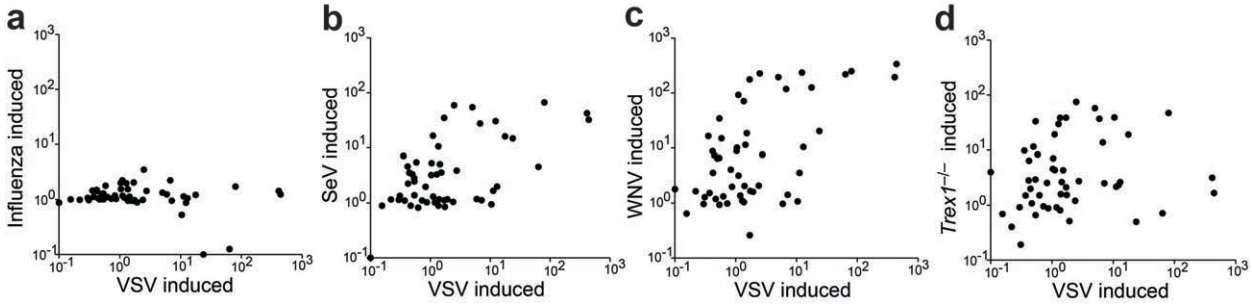
b



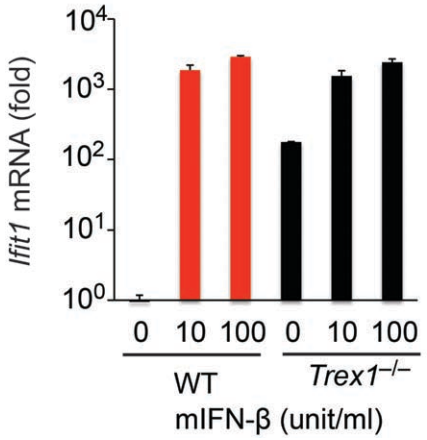
c



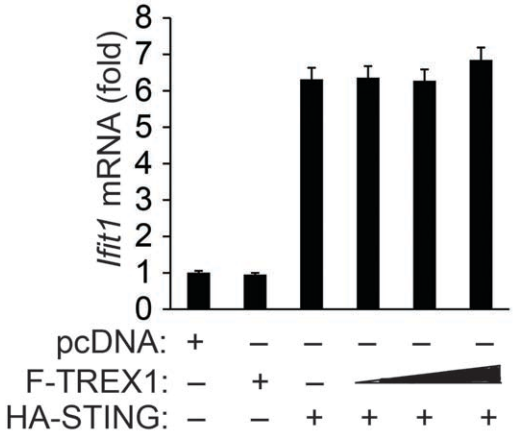
Supplementary Figure 5. Ingenuity pathway analysis of genes up-regulated in *Trex1*$^{-/-}$ cells. Gene expression data from RNA-SEQ were analyzed by Ingenuity Pathway Analysis (IPA) software package. (**a**) Top five ranked gene networks. (**b**) Detailed view of the most enriched gene network (#1 in **a**). Numbers below each molecule represent fold up-regulation in *Trex1*$^{-/-}$ cells compared to WT cells. Molecules in red were up-regulated more than 10-fold. (**c**) Top ten ranked canonical pathways. Black bars show the *p*-value of each pathway. Red line represents percentage of genes (Ratio) within each pathway that were up-regulated in *Trex1*$^{-/-}$ dataset. Red arrows highlight two innate immune pathways that are up-regulated in *Trex1*$^{-/-}$ cells and have high ratio of hits.



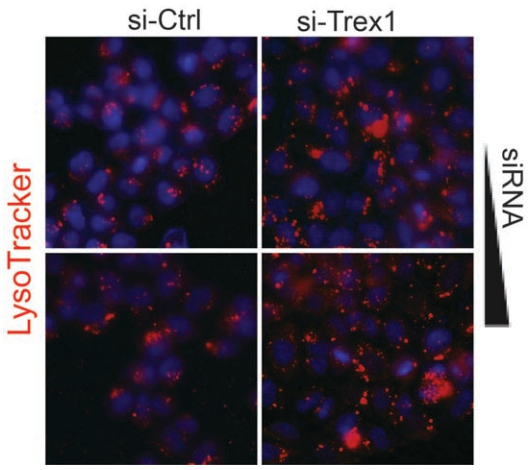
Supplementary Figure 6. ISG induction signature in infected WT cells (a-c) and uninfected *Trex1*$^{-/-}$ cells (d). Each dot represents a gene from **Fig. 3a**: the x-axis value is fold-increase after VSV infection in WT MEFs, and the y-axis value is fold-increase after influenza (**a**), Sendai (**b**) or West Nile virus (**c**) infection in WT MEFs as indicated on the y axis, or fold-increase in uninfected *Trex1*$^{-/-}$ MEFs (**d**). All compared to uninfected WT MEFs.



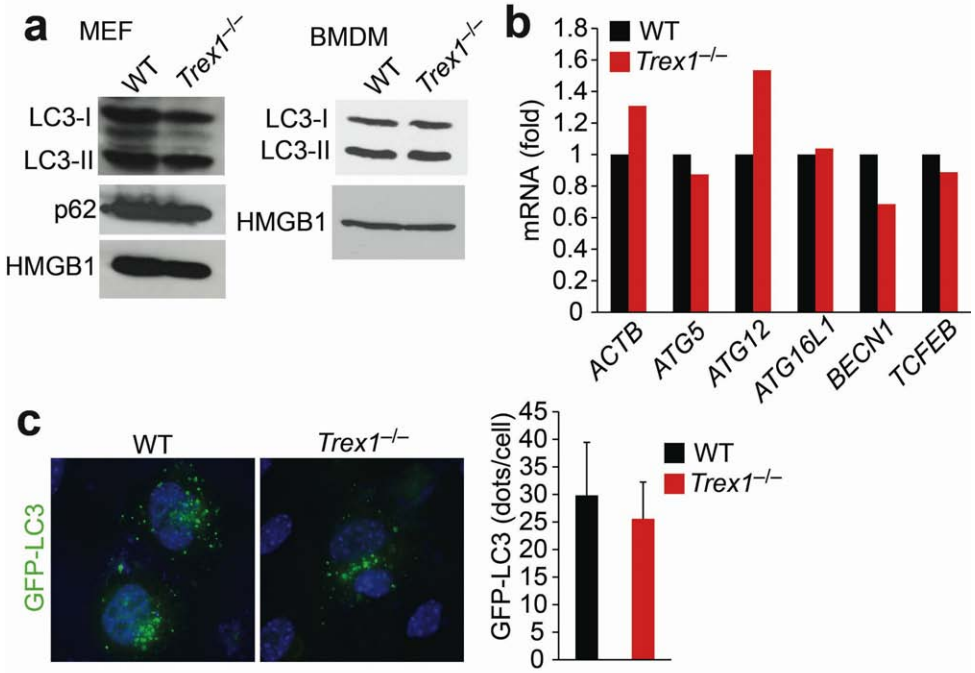
Supplementary Figure 7. IFN-β induces *Ifit1* expression in WT and *Trex1*$^{-/-}$ MEFs. WT and *Trex1*$^{-/-}$ MEFs were treated with recombinant mIFN-β at indicated dose for 6 h. *Ifit1* mRNA level was measured by qRT-PCR. Untreated WT sample was normalized to 1. Data are representative of two independent experiments (error bars, s.d.).



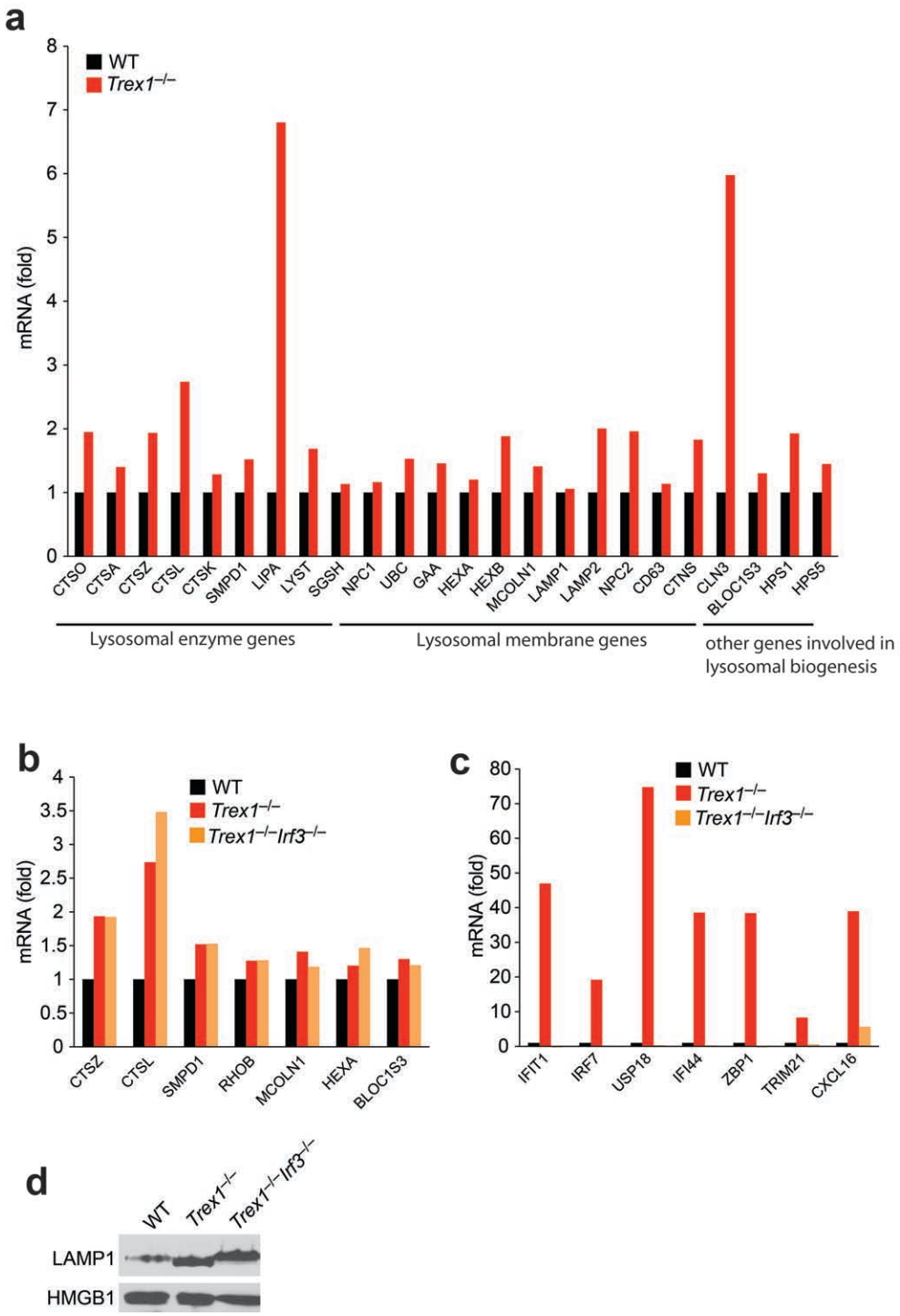
Supplementary Figure 8. Trex1 does not directly inhibit STING-mediated activation of *Ifit1* in 293T cells. 293T cells were transfected with plasmids expression Flag-TREX1 or HA-STING as indicated. Twenty-four hours after transfection, *Ifit1* mRNA was measured by qRT-PCR. pcDNA transfected sample was normalized to 1. Data are representative of three independent experiments (error bars, s.d.).



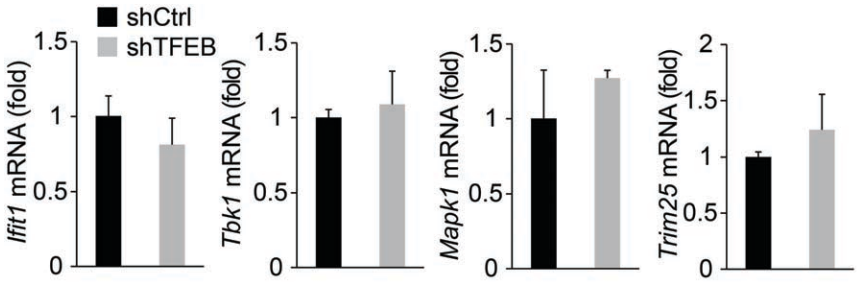
Supplementary Figure 9. Trex1 knockdown in HeLa cells induces lysosomes. HeLa cells were transfected with Ctrl or Trex1 siRNA for 72 h and stained with LysoTracker Red. Two doses of siRNA were used, 20 pmole and 40 pmole. Representative images from three independent experiments are shown.



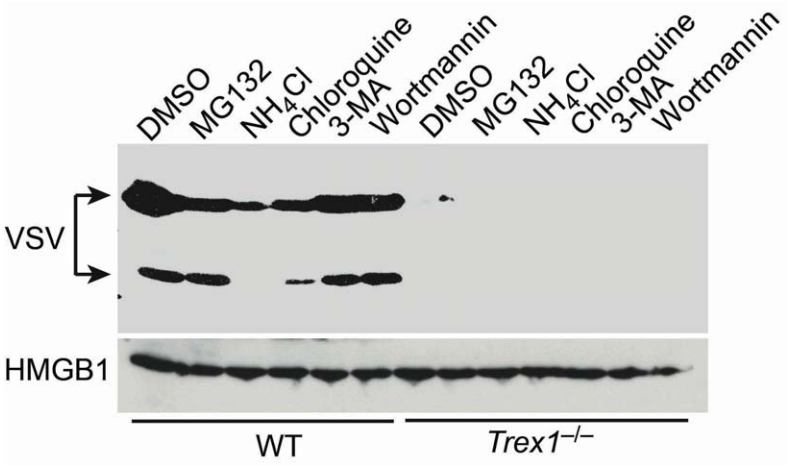
Supplementary Figure 10. Autophagsome formation in WT and *Trex1*$^{-/-}$ cells. (a) Western blot analysis of endogenous LC3, p62 proteins in WT and *Trex1*$^{-/-}$ MEFs and BMDMs. HMGB1 serves as loading controls. Representative blots from three independent experiments are shown. (**b**) Control (*ACTB*) and autophage-related gene expression values determined by RNA-SEQ (**Fig. 3**). WT values were normalized to 1. (**c**) Fluorescence microscopy analysis of GFP-LC3 dot formation in WT and *Trex1*$^{-/-}$ MEFs. Cells grown on slides were transfected with GFP-LC3 plasmids and fixed 24 h post transfection. Numbers of GFP-LC3 dot per cell are shown on the right. Averages of 12 cells are shown (error bars. s.d.).



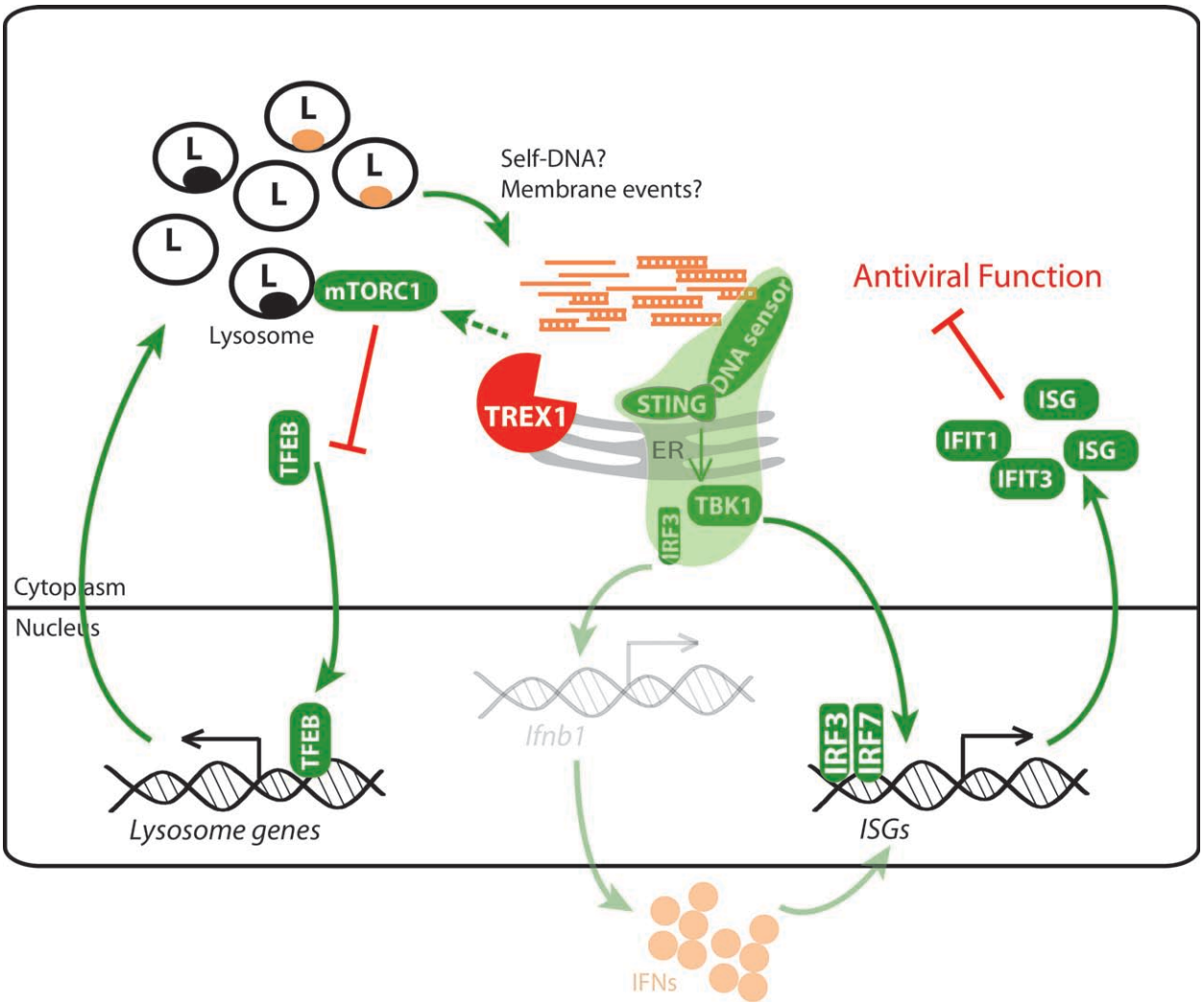
Supplementary Figure 11. Genes related to lysosomal biogenesis are up-regulated in *Trex1*$^{-/-}$ cells. Gene expression values were determined by RNA-SEQ as in **Fig. 3**. WT values were normalized to 1. (**a**) lysosomal gene expression values comparing WT and *Trex1*$^{-/-}$. (**b-c**) lysosomal gene (**b**) or ISGs (**c**) expression values comparing WT, *Trex1*$^{-/-}$ and *Trex1*$^{-/-}$*Irf3*$^{-/-}$. (**d**) Western blot analysis of LAMP1 protein level in WT, *Trex1*$^{-/-}$ and *Trex1*$^{-/-}$*Irf3*$^{-/-}$ MEFs. A representative blot from three independent experiments is shown.



Supplementary Figure 12. TFEB knockdown does not affect innate immune gene expression. MEFs stably expressing shCtrl or shTFEB were used for qRT-PCR analysis of indicated genes. Data are representative of three independent experiments (error bars, s.d.).



Supplementary Figure 13. Effects of various inhibitors on VSV replication in WT and *Trex1*$^{-/-}$ cells. Western blot analysis of VSV proteins in WT and *Trex1*$^{-/-}$ MEFs treated with indicated drugs for 1 h and infected with VSV for 24 h. MG132 was used at 2.5 uM, NH_4Cl at 20 mM, chloroquine at 50 uM, 3-MA at 1mM, wortmannin at 10 uM. A representative blot from two independent experiments is shown.



Supplementary Figure 14. A model showing how TREX1 regulates lysosomal biogenesis and IFN-independent activation of antiviral genes.

Supplementary Table 1. DNA oligos used in this study. All oligos were purchased from Sigma.

Oligo name	**Forward and reverse oligo sequence**
Mouse *Gapdh*	TTCACCACCATGGAGAAGGC, GGCATCGACTGTGGTCATGA
Mouse *Ifnb*	CTGCGTTCCTGCTGTGCTTCTCCA, TTCTCCGTCATCTCCATAGGGATC
VSV *G*	CAAGTCAAAATGCCCAAGAGTCACA, TTTCCTTGCATTGTTCTACAGATGG
VSV *M*	TATGATCCGAATCAATTAAGATATG, GGGACGTTTCCCTGCCATTCCGATG
Influenza *NS1*	TCGAGACAGCCACACGTGCTGGAAA, AAGAGGGCCTGCCACTTTCTGCTTG
SeV *P*	TGTTATCGGATTCCTCGACGCAGTC, TACTCTCCTCACCTGATCGATTATC
WNV *Env*	TCACGCATCTCTCCACCAAAG, GGGTCAGCACGTTTGTCATTG
Mouse *Ifit1*	GAACCCATTGGGGATGCACAACCT, CTTGTCCAGGTAGATCTGGGCTTCT
Mouse *Ifit2*	ATGAGTTTCAGAACAGTGAGTTTAA, AACTGGCCCATGTGATAGTAGACCC
Mouse *Ifit3*	TGGCCTACATAAAGCACCTAGATGG, CGCAAACTTTTGGCAAACTTGTCT
Mouse *Irf7*	ATGCACAGATCTTCAAGGCCTGGGC, GTGCTGTGGAGTGCACAGCGGAAGT
Mouse *Ifitm3*	GAGGTGGCTGAGATGGGGGCACCG, CTCCAGTCACATCACCCACCATCTT
Mouse *Stat1*	AAGGTGAAGCCAATGGTGTGGCGAA, CCGATGCAGGCGCTCTGCTGCCTTC
Mouse *Stat2*	ACAGGATGTCTTCAGCTTCAGATAC, CACTCGTCCAGCTTGGGCAGCAATA
Mouse *Ifna4*	CTTTCCTCATGATCCTGGTAATGAT, AATCCAAAATCCTTCCTGTCCTTC
Mouse *Ctsa*	GACTCCAAGCACTTCCACTACTGGT, CTGGCTGGATCAGAAAGGGGCCGTG
Mouse *Sgsh*	CCCTGTCCCGCCACAGCCTTATCTT, GAGTTGAAGTGATGCACATCCTGGT
Mouse *Lamp1*	TAATGGCCAGCTTCTCTGCCTCCTT, AGGCTGGGGTCAGAAACATTTTCTT
Mouse *Mcoln1*	CCCACAGAAGAGGAAGACCTCCGCC, AGAGAATGAGCTGCACAGTGACCAC
Mouse *Tpp1*	GCTGGGTGTCCCTGGGCCGCGTGGA, AGGGTTAGGTACTTTCCATATTGAG
Mouse *Tcfeb*	GAGCTAACAGATGCTGAGAGCAGAGC, GCATCCTCCGGATGTAATCCACAGA

Mouse *Tbk1*	CCAGTGGATGTTCAAATGAGAGAAT, TCTAGAACAGTGTATAAACTCCCAC
Mouse *Mapk1*	CTGCTTATGATAATCTCAACAAAGT, TGCCCGGATGATGTCATTGATGCCA
Mouse *Trim25*	AATGTCGCAAAGTGTACCAGGTGCG, ACGAGGCACGTCTTCACTGCGATCT

Supplementary Table 2. siRNA oligos used in this study. All oligos were purchased from Sigma.

Oligo name	**Sequence**
Control	UAGCGACUAAACACAUCAA
Trex1	GCUACAGCCUGGGCAGCAU, CAGGGAAUGGUUCGAGGAA, CACACAACGGUGACCGCUA
IFIT1	GAAAUGAACCCUGCAUUCU, CAACAAAUCUCCCAACUGA
IFITM3	CACGGAUCGGCUUCUGUCA, GCACCUUGGUCCUCAGCAU
STAT1	CUCAGAACACUCUGAUUAA, CACAGUAUAAACACGAAUU
STAT2	GAAUCAGGCUCAAAGAGCU, GUGAUUAUUUCUAACAUGA
IRF3	CAAGGUUGUUCCUACAUGU, GUCCUCAGAUCUGGCUAUU
IRF7	GGAAAUUGCCCUCGAUGUU, CACCUAAUUUACUAGAGCU
TFEB	CAACAGUCCCAUGGCCAUG, CCAACCUGUCCAAGAAGGA
TBK1	CGGAAGAGUGGAUGAGAAA
STING	UCAAUCAGCUACAUAACAA
IFI204	UUAGUUUACUGCCUGGUUC
RIG-I	CAAGAAGAGUACCACUUAA
MAVS	GAUCAAGUGACUCGAGUUU
mTOR	GGAUCAACCACCAGCGCUA, CUGACUACGCCUCCCGCAU