Supplemental Figure Legends

Figure S1. NLRX1 inhibits VSV-induced IFN-I response. (A) WT and *Nlrx1*$^{-/-}$ MEFs were harvested for RNA extraction. *Nlrx1* mRNA level was evaluated by RT-PCR. 18s mRNA level was assessed as internal control. (B-C) WT and *Nlrx1*$^{-/-}$ MEFs were untreated or infected with VSV (MOI of 0.1) for 12 hr. Transcripts of inflammatory cytokines including *Il6*, *Tnfa*, *Ifna4* and *Ifnb1* were assessed by real time PCR. Protein production of IL-6 and IFN-β was measured by ELISA. Results of all experiments are representative of at least 2-3 repeats. Values are expressed as mean ± s.d. * $P < 0.05$, versus controls. (D) NLRX1-interacting proteins were annotated on their subcellular localization based on the Gene Ontology database. The number of proteins that reside in the same compartment was divided by the total number of proteins to calculate the percentage. (E, F) MS/MS spectrum of the peptides AEAGDNLGALVR and LLDAVDTYIPVPAR were captured from those derived from the interacting partners with NLRX1 full-length protein. (G, H) MS/MS spectrum of the peptides ELLTEFGYK and AEAGDNLGALVR were captured from those derived from the interacting partners with NLRX1ΔLRR protein. These peptides are further shown in Table S1.

Figure S2. TUFM is ubiquitously expressed in multiple cell and tissue types and confirmation of TUFM knockdown by shRNA. (A-B) The mRNA expression data of human TUFM is made available by the BioGPS project (http://biogps.gnf.org/), which is funded by the Genomics Institute of the Novartis Research Foundation. TUFM was used as the key identification word. (C) HEK293T cells were transfected with siRNA pool targeting TUFM or control siRNA pool. Samples were analyzed by SDS-PAGE and

subsequent immunoblotting for TUFM. (D) HEK293T cells were transduced with lentiviruses containing either tetracycline-inducible TRIPZ™ control vector or shRNA targeting TUFM. 1 μg/ml doxycycline was added to cell culture and incubated for 48 hr before cells were harvested for protein extraction. Samples were subjected to SDS-PAGE and immunoblotting for TUFM.

Figure S3. NLRX1 is essential for VSV-induced autophagy in MEFs. WT and *Nlrx1*$^{-/-}$ MEFs were infected with VSV (MOI of 0.1 or 1.0) for 12 hr in the absence or presence of bafilomycin A1 (100 nM). Protein samples were immunoblotted for LC3B. Densitometry analysis to quantify ratio of LC3B-II to β-actin is shown at the bottom.

Figure S4. Protocol for subcellular fractionation. For each cell fractionation experiments, 5×10^7 cells were lysed in hypotonic lysis buffer with a dounce homogenizer. Then serial centrifugation was carried out to separate each compartment according to the scheme.

Table S1. The peptides sequences matching TUFM were identified in both NLRX1 full-length group and NLRX1ΔLRR mutant group. Peptides were eluted from NLRX1 or NLRX1ΔLRR groups. Two unique peptides with at least five consecutive amino acids sequences which matched sequences in IPI (International Protein Index) database were found in each eluate. The ion scores for all peptides shown are equal or greater than 39, which indicates a greater than 95% possibility that the peptides detected represent TUFM protein ID in the IPI database.