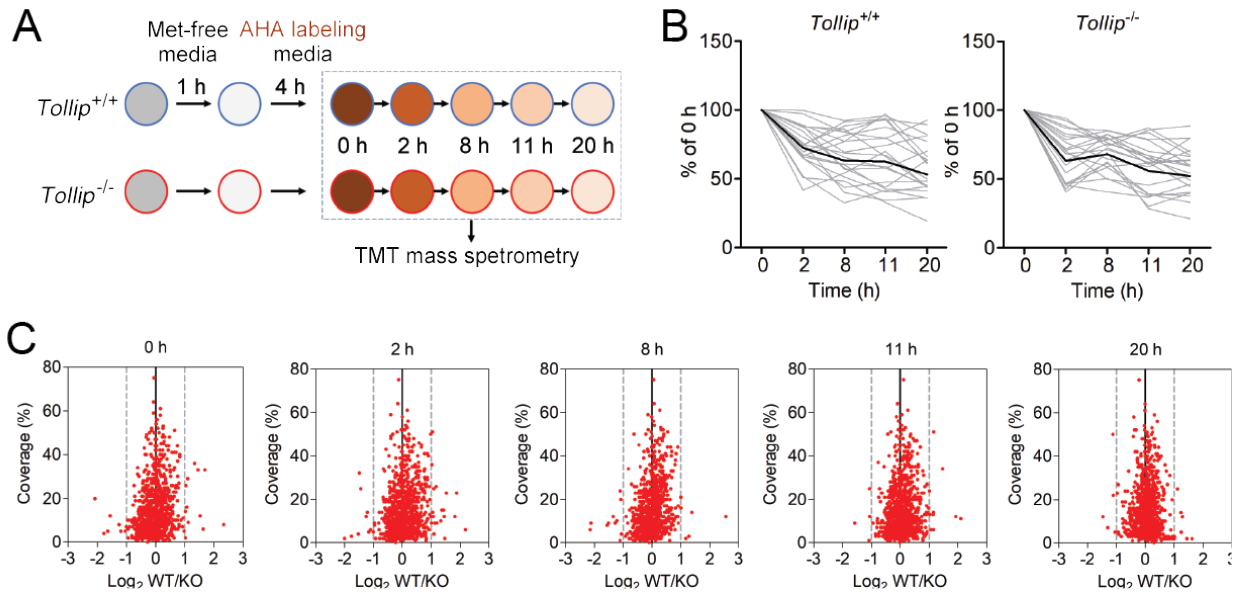
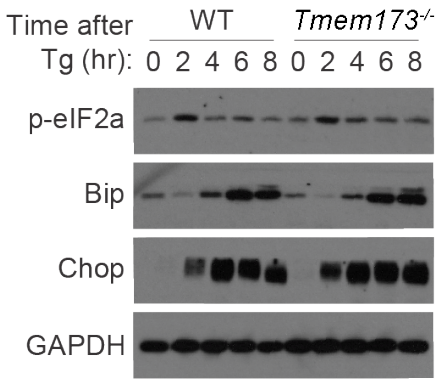
SUPPLEMENTARY INFORMATION



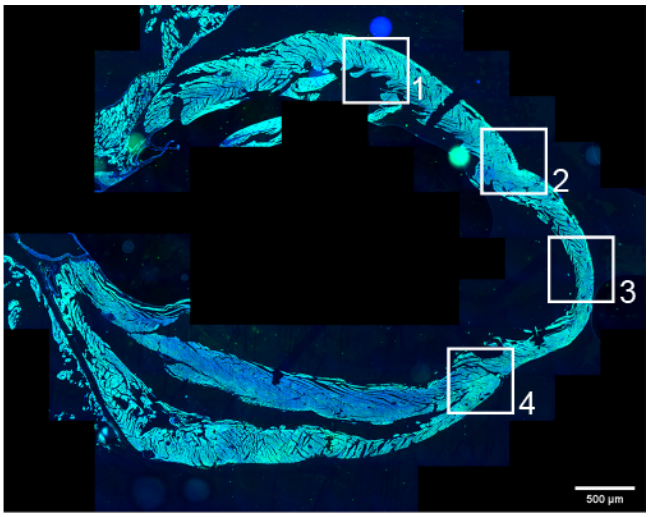
Supplementary Figure 1: *Tollip*$^{-/-}$ does not cause global protein instability in cells.

Global proteomic analysis of protein stability in *Tollip*$^{+/+}$ and *Tollip*$^{-/-}$ MEFs. Cells were pause labeled with Click-iT® AHA (L-azidohomoalaine) for 4 h, followed by immunoprecipitation of AHA-labelled proteins at various times after labeling. Labeled protein samples were analyzed by quantitative Tandem Mass Tag mass spectrometry (TMT-MS). See Methods for more details. **(A)** a schematic diagram of the experimental design. **(B)** Average protein turnover in *Tollip*$^{+/+}$ and *Tollip*$^{-/-}$ MEFs. Top 20 proteins that are actively turned over (low value at 20 h) in *Tollip*$^{+/+}$ and *Tollip*$^{-/-}$ MEFs were presented as gray lines and the average is presented as a black line. Protein level at each time point was normalized to time 0 as 100%. **(C)** Volcano plots of WT/KO ratio for each protein at indicated time point. Note that very few proteins are showing more than 2-fold increase or decrease in stability (outside of the gray dotted line).



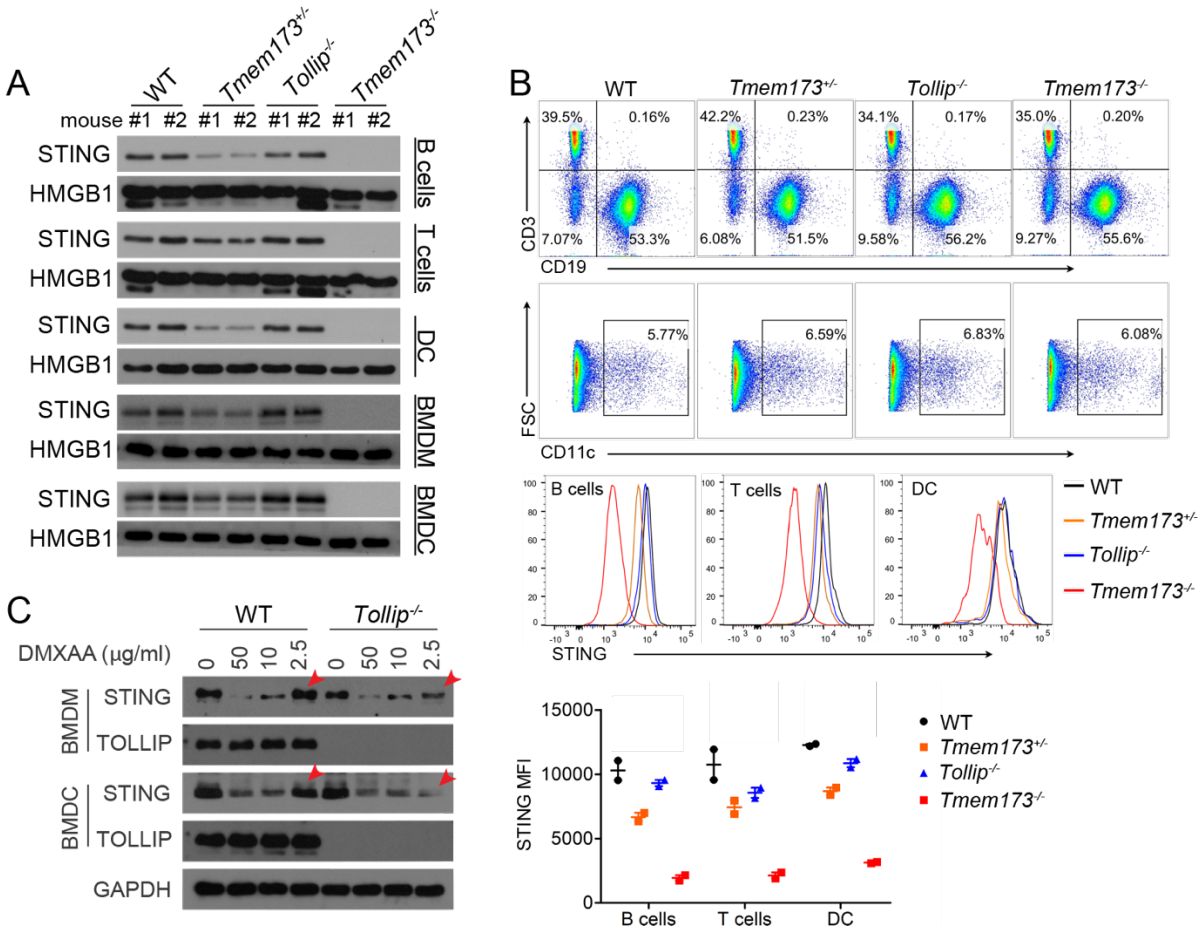
Supplementary Figure 2: WT and *Tmem173*$^{-/-}$ show similar Tg-induced ER stress and the UPR.

Immunoblot analysis of UPR proteins in WT and *Tmem173*$^{-/-}$ MEFs treated with thapsigargin (Tg, 500 nM) for indicated amount of time (top). This experiment was repeated twice.



Supplementary Figure 3. Heart tissue area sampled for STING protein quantitation.

A representative image showing 4 areas of the heart chosen for quantifying STING fluorescent signal. Twenty-five region-of-interests (ROIs) were randomly chosen from each area to get a total of 100 individual data points per genotype presented in **Figure 9D**. Scale bar, 500 µm.



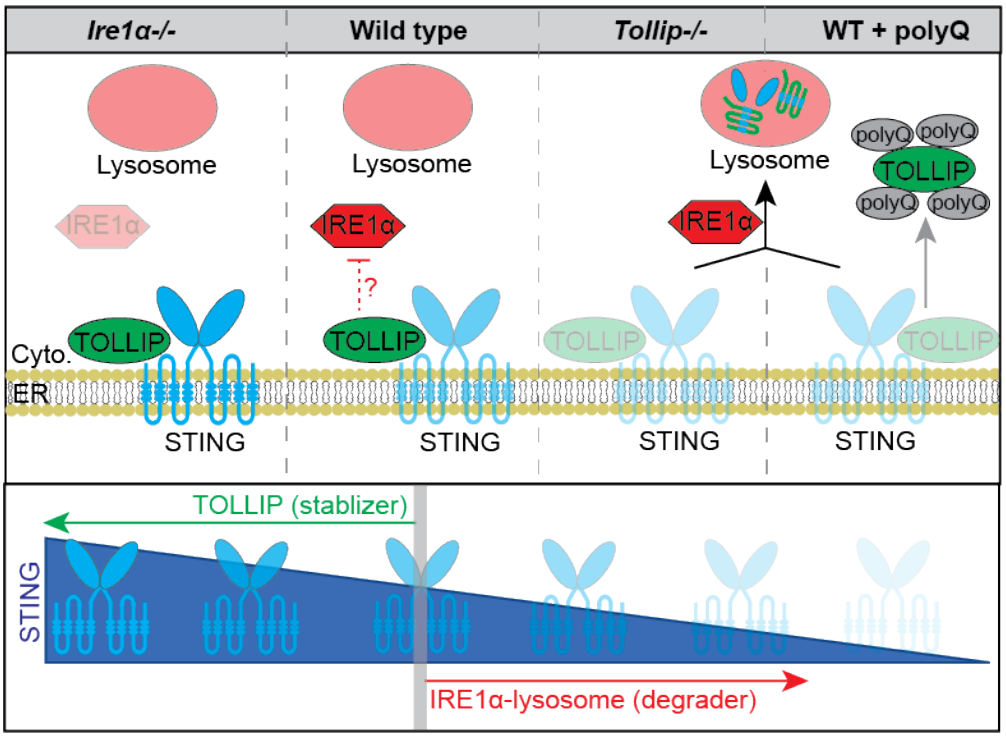
Supplementary Figure 4. STING protein is unstable in *Tollip*$^{-/-}$ immune cells.

(A) immunoblot analysis of STING protein in indicated immune cells (right) isolated from wild type (WT), *Tollip*$^{-/-}$ and *Tmem173*$^{+/-}$ and *Tmem173*$^{-/-}$ spleen or derived from bone merrow (BMDM, BMDC).

(B) FACS analysis of STING protein in indicated immune cells isolated from wild type (WT), *Tollip*$^{-/-}$ and *Tmem173*$^{+/-}$ and *Tmem173*$^{-/-}$ mice. Gating strategy is showing on top. A representative set of STING FACS plots are showing in the middle. Quantitation of STING protein mean fluorescent intensity (MFI) from two experiments is showing on the bottom.

(C) immunoblot analysis of STING protein in *Tollip*$^{+/+}$ (WT), *Tollip*$^{-/-}$ BMDM or BMDCs (indicated on left) after different dose of DMXAA stimulation. Red arrow denotes low-dose DMXAA treatment that reveals that STING protein is unstable in *Tollip*$^{-/-}$ cells.

Data are representative of two independent experiments.



Supplementary Figure 5: Graphical abstract.

Top, STING protein is stabilized by TOLLIP via direct interaction on the ER (wild type panel in the middle). In *Tollip*$^{-/-}$ cells or cells expressing polyQ proteins, STING protein decreases due to degradation by the IRE1α-lysosome pathway (right panels). In *Ire1α*$^{-/-}$ cells, STING protein increases (more stabilized). Our study suggest that homeostatic regulation of STING is mediated by active tug-of-war between TOLLIP and IRE1α-lyosome. **Bottom,** tug-of-war between TOLLIP the 'stabilizer' and IRE1α-lysosome the 'degrader' that together set the threshold of resting-state STING protein level to maintain tissue homeostasis.