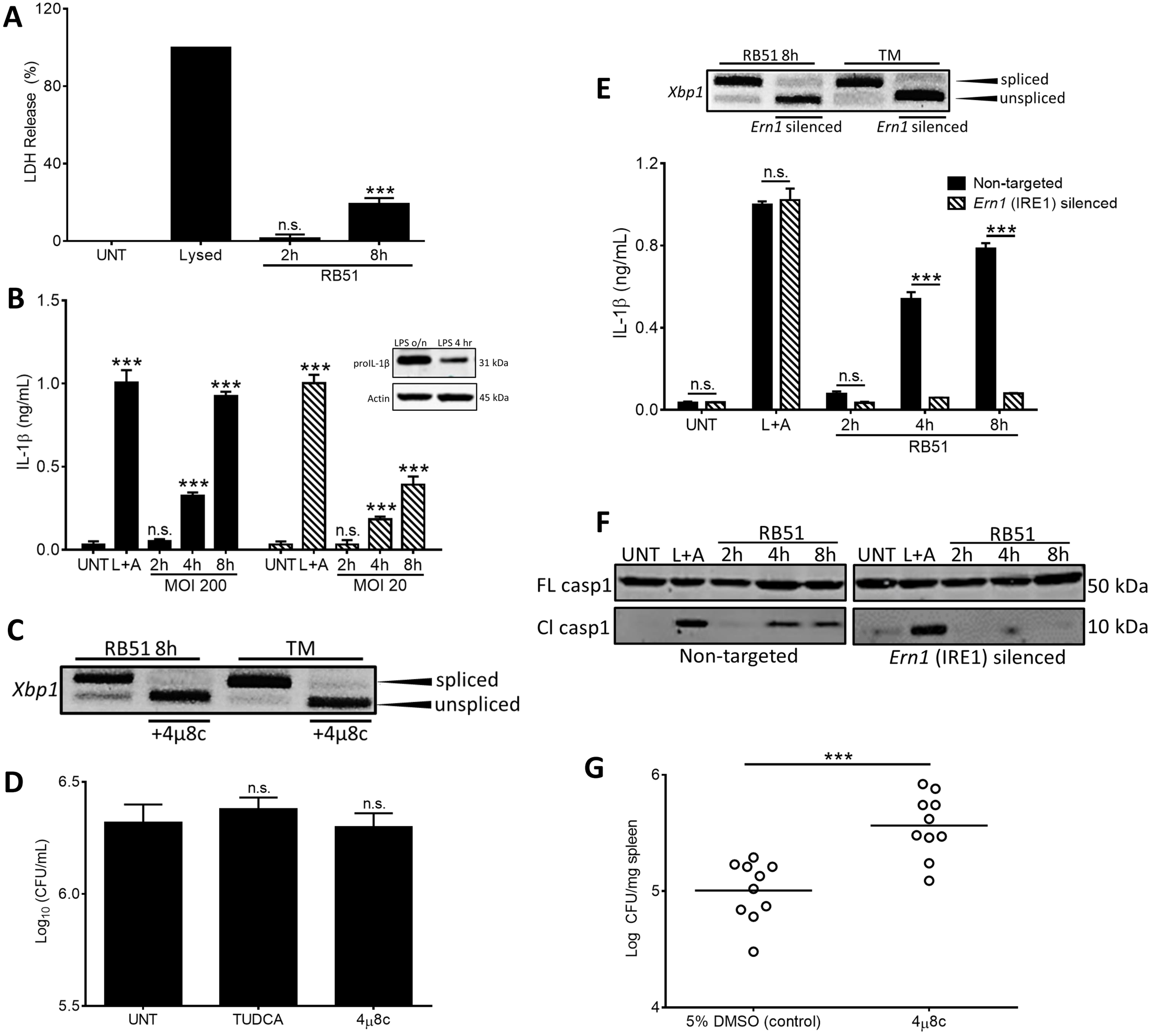

Figure S1: RB51 infection induces IRE1 dependent IL1β production and bacterial killing without substantial cell death (associated with Figure 1). (A) LDH release of RB51-infected (MOI 200) BMDM. Lysed (Triton-X100 treated) and untreated (UNT) cells served as positive and negative controls respectively. (B) IL-1β levels in macrophages treated with LPS+ATP (positive control of IL-1β, 200 ng/mL and 1mM respectively) or infected with RB51-infected at MOI 200 or 20. Inset is an immunoblot of proIL-1β protein levels in cell treated with LPS (200 ng/ml) overnight (o/n) or for 4 hr prior to ATP treatment – LPS o/n induced robust proIL-1β and served as a positive control for IL-1β production. (C) Immunoblot demonstrating 4μ8c efficacy of inhibiting IRE1-induced *Xbp1* splicing in TM (tunicamycin, 10 μg/mL) treated and RB51-infected BMDM. (D) BMDM were pretreated with TUDCA (chemical chaperone, 300 μM) or 4μ8c (IRE1 inhibitor, 50 μM) for one (1) hour prior to infection. These pretreated BMDM were then infected with RB51 (MOI 200). One (1h) p.i BMDMs were lysed to enumerate intracellular CFU. (E) IL-1β levels and (F) caspase-1 cleavage in RB51-infected non-targeted and *Ern1* (IRE1) silenced RAW264.7 macrophages. Immunoblots in (F) are representative of n≥3 independent experiments that were performed and imaged in parallel with identical parameters using a LiCor Odyssey imaging system. Full length caspase-1 serves as loading controls. The inset demonstrates the *Xbp1* splicing in TM (tunicamycin, 10 μg/mL) treated and RB51-infected *Ern1* (IRE1) silenced BMDM. (G) Mice were intraperitoneally (i.p.) injected with *Brucella abortus* RB51 (1 x 10^8 CFU). Mice were treated with 5% DMSO (n = 10) or 4μ8c (n = 10) on day 0 – 3 post infection. The spleens were collected 3 days post infection and bacterial numbers (Log CFU/mg spleen) were measured. The data were pooled from 2 separate experiments. Error bars represent mean ± SD of n≥3 independent experiments. *** represent p-value <0.0001 respectively. n.s. = not significant.

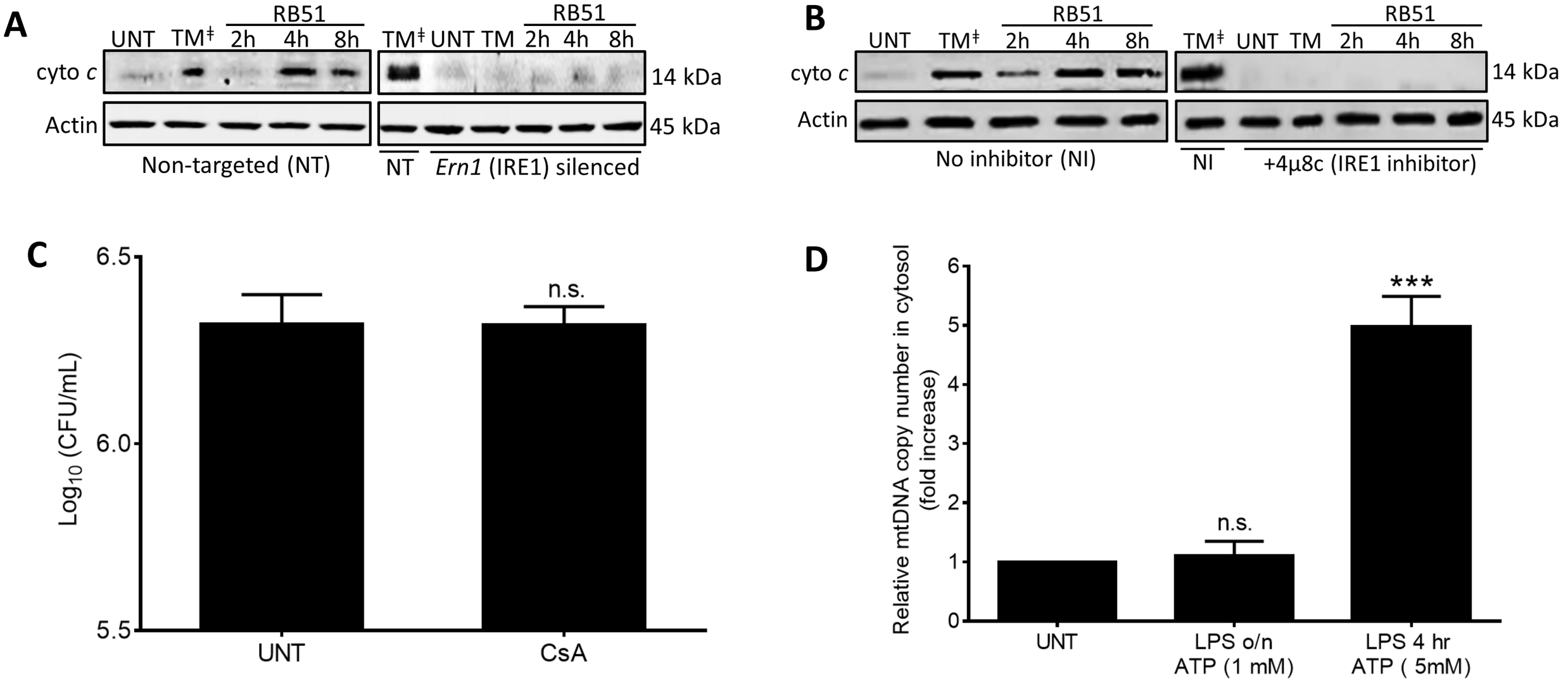

Figure S2: IRE1 mediates RB51-induced mitochondrial damage (associated with Figure 2). Immunoblot analysis of cytochrome *c* (cyto *c*) in cytosolic extracts from RB51-infected BMDM in (A) non-targeted or *Ern1* (IRE1) silenced RAW264.7 macrophages or in (B) the absence or presence of 4μ8c (IRE1 inhibitor, 50 μM) – TM‡ denotes the same control TM-treated sample. The blots were probed with anti-actin antibody as a loading control. Immunoblots are representative of n≥3 independent experiments that were performed and imaged in parallel with identical parameters using a LiCor Odyssey imaging system. (C) BMDM were pretreated with cyclosporin A (CsA, inhibitor of MPTP opening 10 μM) for 1h prior to infection. These pretreated BMDM were then infected with RB51 (MOI 200) and 1h p.i BMDM were lysed to enumerate intracellular CFU. (D) Amount of mtDNA released into the cytosol when treated with two different L+A treatment protocols – LPS o/n did not trigger mtDNA release and served as an control for inflammasome activation independent of mitochondrial damage. UNT and TM represent untreated and tunicamycin (10 μg/mL, positive control for ER stress-induced mitochondrial damage) respectively. *** represent p-value <0.0001 respectively. n.s. = not significant.

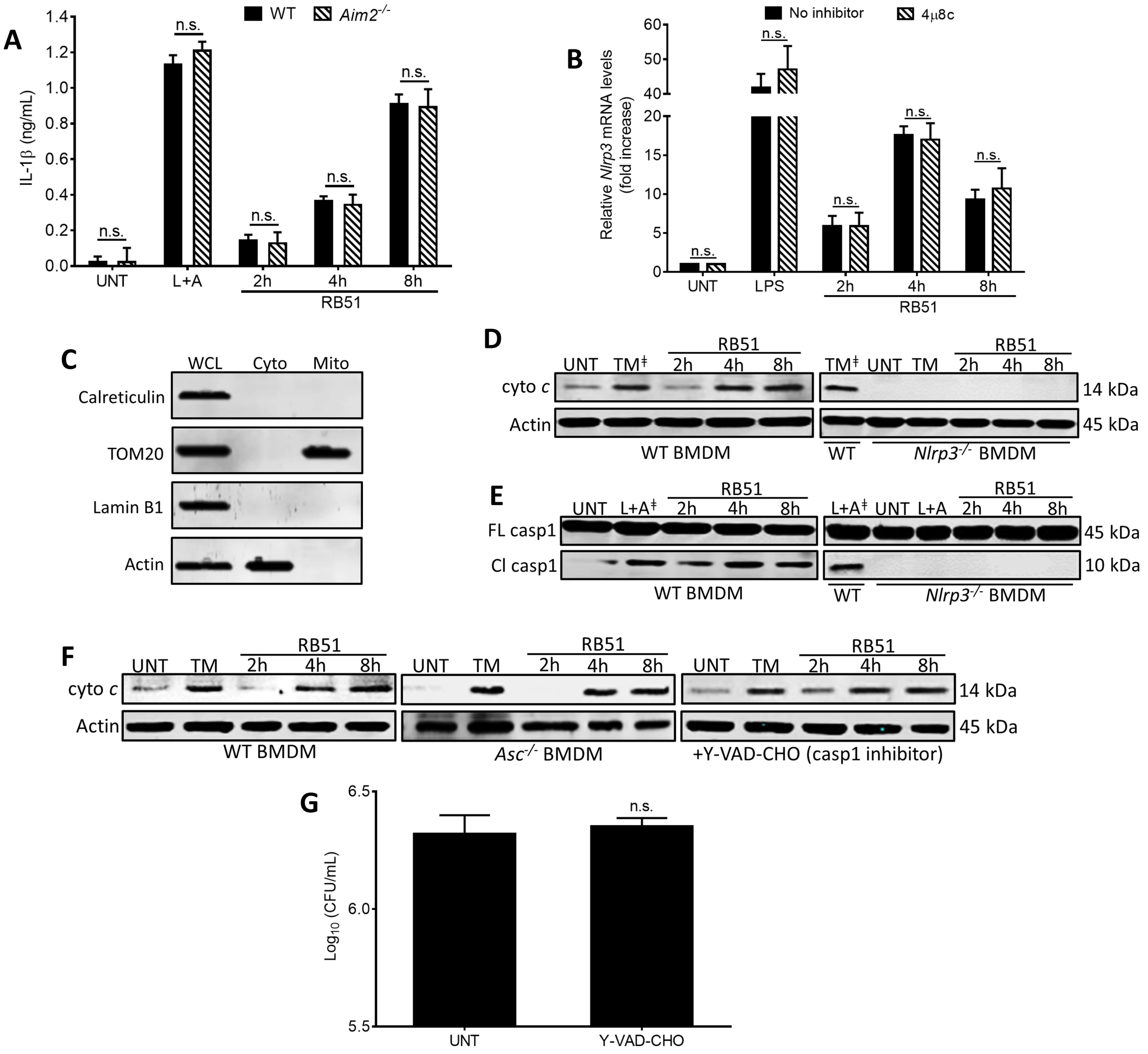

Figure S3: NLRP3, not AIM2, mediates mitochondrial damage independent of ASC and caspase-1 (associated with Figure 3). (A) ELISA analysis of IL-1β levels in the supernatant of WT and *Aim2*$^{-/-}$ RB51-infected BMDM. (B) qRT-PCR analysis of *Nlrp3* transcript levels in RB51-infected BMDM in absence or presence of 4μ8c. (C) Whole Cell lysate (WCL), cytosolic fraction (Cyto), and mitochondrial fraction (Mito) were assessed for the presence of different cellular markers: Calreticulin (ER marker), TOM20 (mitochondrial marker), Lamin B1 (nuclear marker), and Actin (cytosolic marker). (D) Immunoblot analysis of cytochrome *c* (cyto *c*) in cytosolic extracts from RB51-infected WT and N*lrp3*$^{-/-}$ BMDM – TM‡ indicates duplicate lanes of the same sample. (E) Immunoblot analysis of caspase-1 in RB51-infected WT and *Nlrp3*$^{-/-}$ BMDM – L+A‡ indicates duplicate lanes of the same sample. (F) Immunoblot of cytochrome *c* in WT, *Asc*$^{-/-}$, and Y-VAD-CHO (caspase-1 inhibitor, 2 μM) treated BMDM. Actin and full length (FL) caspase-1 serve as a loading control. The cleaved active form (Cl) was detected on the same blot as FL. (G) BMDM were pretreated with Z-YVAD-CHO (caspase-1 inhibitor, 2 μM) for 1h prior to infection. These pretreated BMDM were then infected with RB51 (MOI 200) and 1h p.i BMDM were lysed to enumerate intracellular CFU. Error bars represent mean ± SD of n≥3 independent experiments. n.s. = not significant. UNT (untreated) and LPS+ATP (trigger of IL-1β production, 200ng/mL and 1mM) respectively.

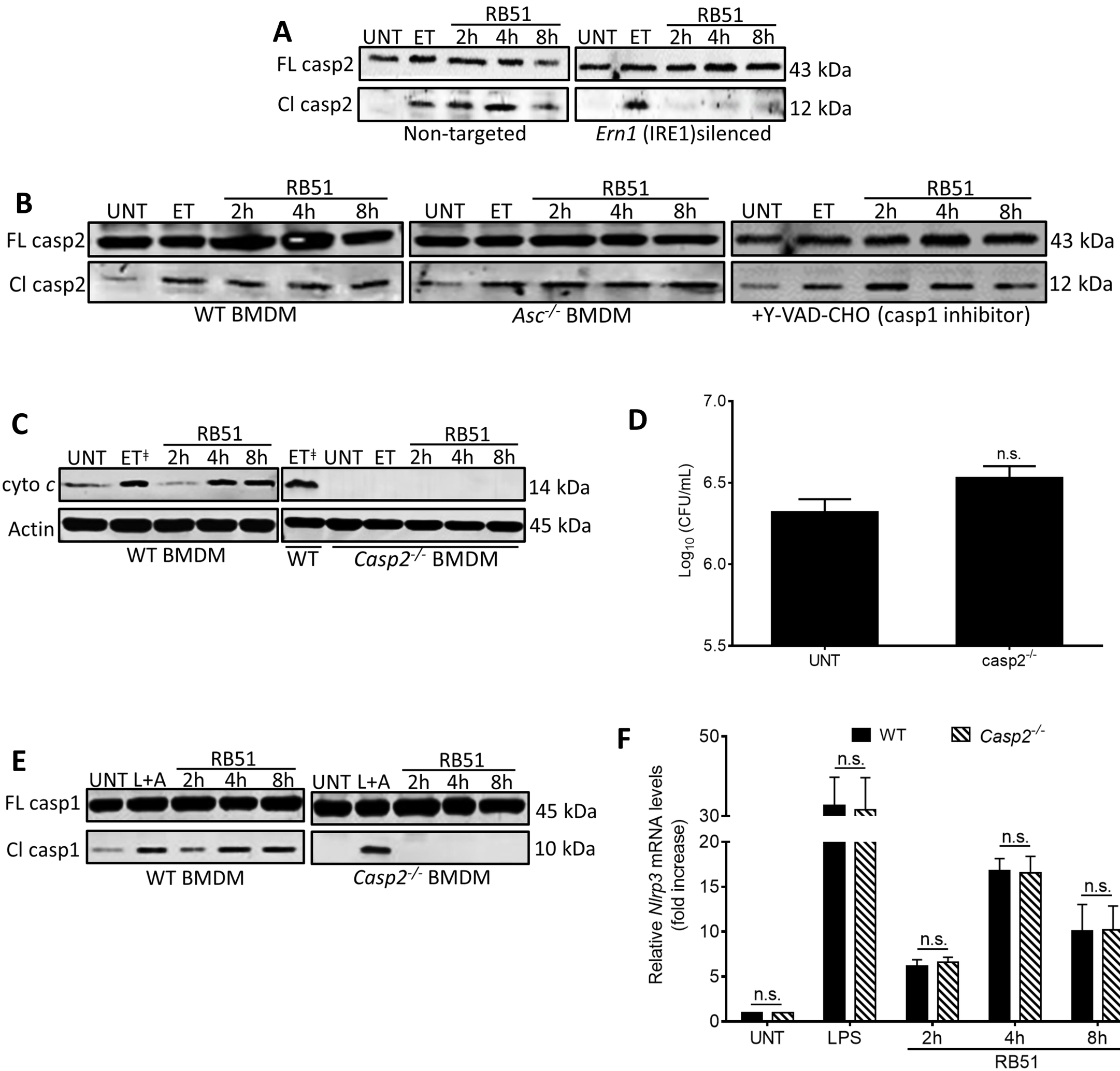

Figure S4: Caspase-2 mediated mitochondrial damage is triggered by IRE1 and NLRP3 not ASC/caspase-1 (associated with Figure 4). Caspase-2 activation in (A) non-targeted (NT) and *Ern1* (IRE1) silenced or (B) WT, *Asc*$^{-/-}$, and Y-VAD-CHO (caspase-1 inhibitor, 2 µM) treated BMDM. (C) cytochrome *c* release in WT and *Casp2*$^{-/-}$ RB51-infected BMDM. (D) Bacterial uptake in WT and *Casp2*$^{-/-}$ BMDM 1h p.i. (E) Immunoblot of caspase-1 cleavage (activation) in WT and *Casp2*$^{-/-}$ RB51-infected BMDM. UNT, ET, and L+A represent untreated, etoposide (inducer of caspase-2 activation, 25 µM), and LPS+ATP (trigger of IL-1β production, 200ng/mL and 1mM) respectively. (F) qRT-PCR analysis of *Nlrp3* transcript levels RB51-infected WT and *Casp2*$^{-/-}$ BMDM. Immunoblots are representative of n≥3 independent experiments that were performed and imaged in parallel with identical parameters using a LiCor Odyssey imaging system. Actin, full length (FL) caspase-2, or full length (FL) caspase-1 serve as loading controls. Error bars represent mean ± SD of n≥3 independent experiments. n.s. = not significant.

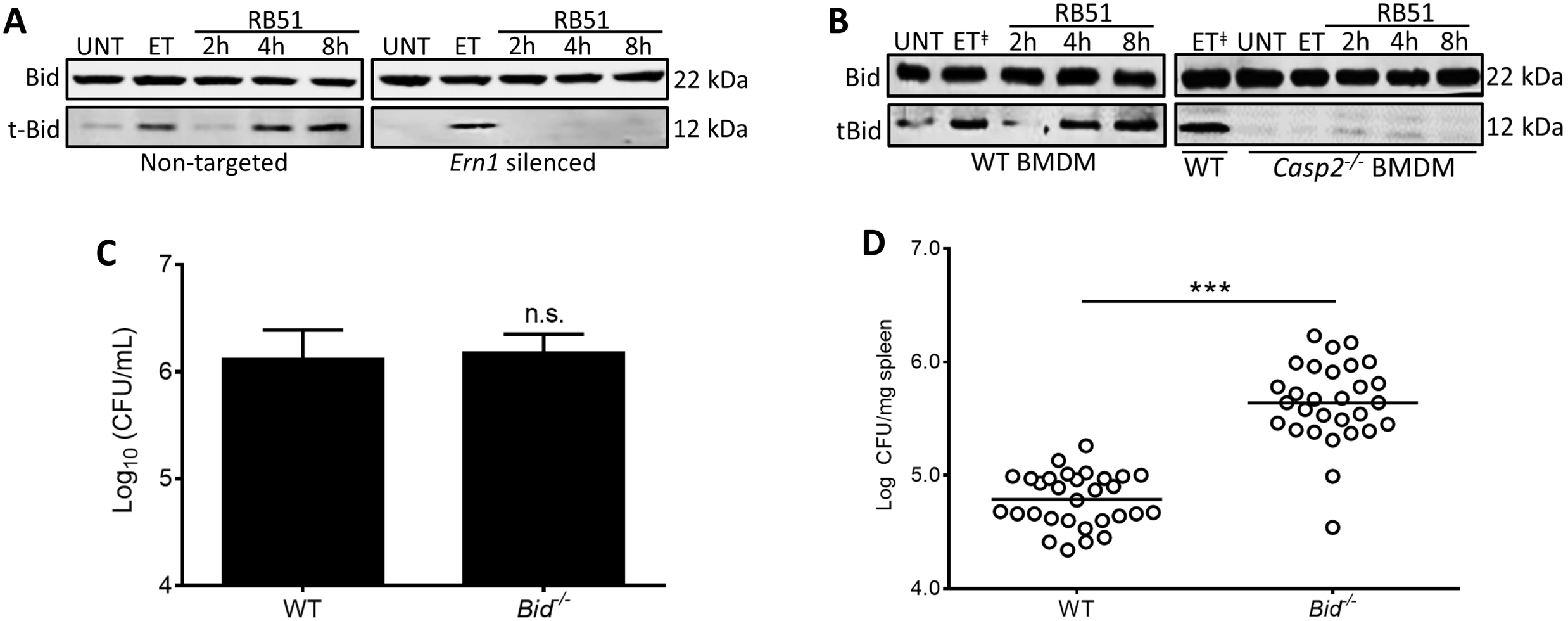

Figure S5: ER stress-induced caspase-2 mediates Bid truncation (associated with Figure 5). Immunoblot analysis of Bid truncation in (A) non-targeted and *Ern1* (IRE1) silenced RAW264.7 macrophages or (B) WT and *Casp2*$^{-/-}$ BMDM infected with RB51 – ET‡ identifies duplicate lanes of the same sample. UNT and ET represent untreated and etoposide (inducer of caspase-2 activation, 25 µM) respectively. (C) Bacterial uptake in WT and *Bid*$^{-/-}$ BMDMs 1h p.i. Immunoblots are representative of n≥3 independent experiments that were performed and imaged in parallel with identical parameters using a LiCor Odyssey imaging system. Full length (FL) Bid serves as a loading control. (D) WT (n=29) and *Bid*$^{-/-}$ (n = 30) mice were intraperitoneally (i.p.) injected with *Brucella abortus* RB51 (1 x 10^8 CFU). The spleens were collected 3 days p.i. and bacterial numbers (CFU/mg spleen) were measured. The data was pooled from 2 separate experiments. *** represent p-value <0.0001.

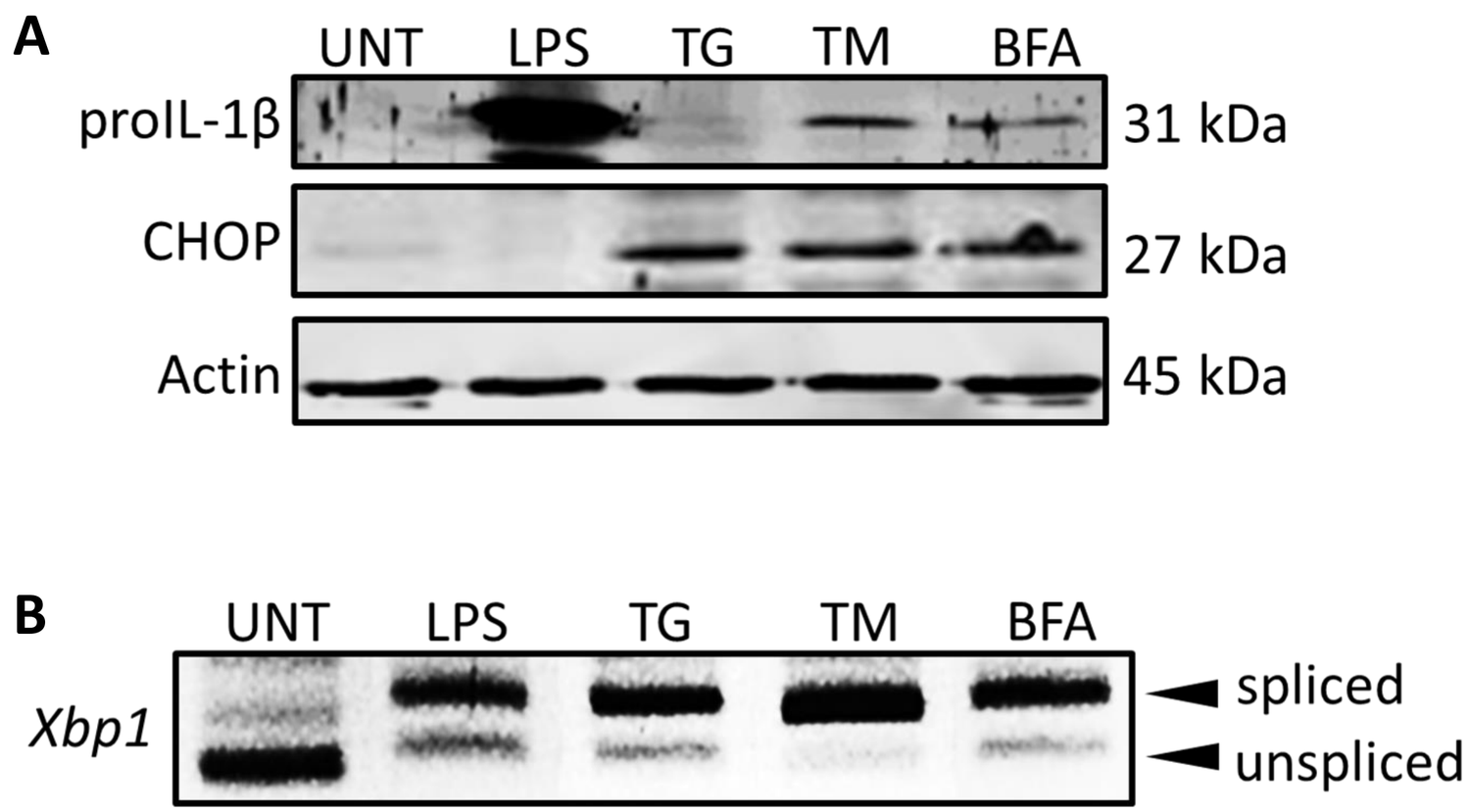

Figure S6: ER stress triggers weak priming of proIL-1β (associated with Figure 6). (A) ProIL-1β and (B) *Xbp1* splicing in BMDM after 4 hour treatment with LPS, as well as ER stress inducers thapsigargin (TG, 10 μM), tunicamycin (10 μg/mL), and brefeldin A (BFA, 20 μM). CHOP serves as a marker of ER stress and actin serves as a loading control. Immunoblots in (A) and inset are representative of n≥3 independent experiments that were performed and imaged in parallel with identical parameters using a LiCor Odyssey imaging system.