# Appendix

To the article entitled

**Host cell egress of Brucella abortus requires BNIP3L-mediated mitophagy**

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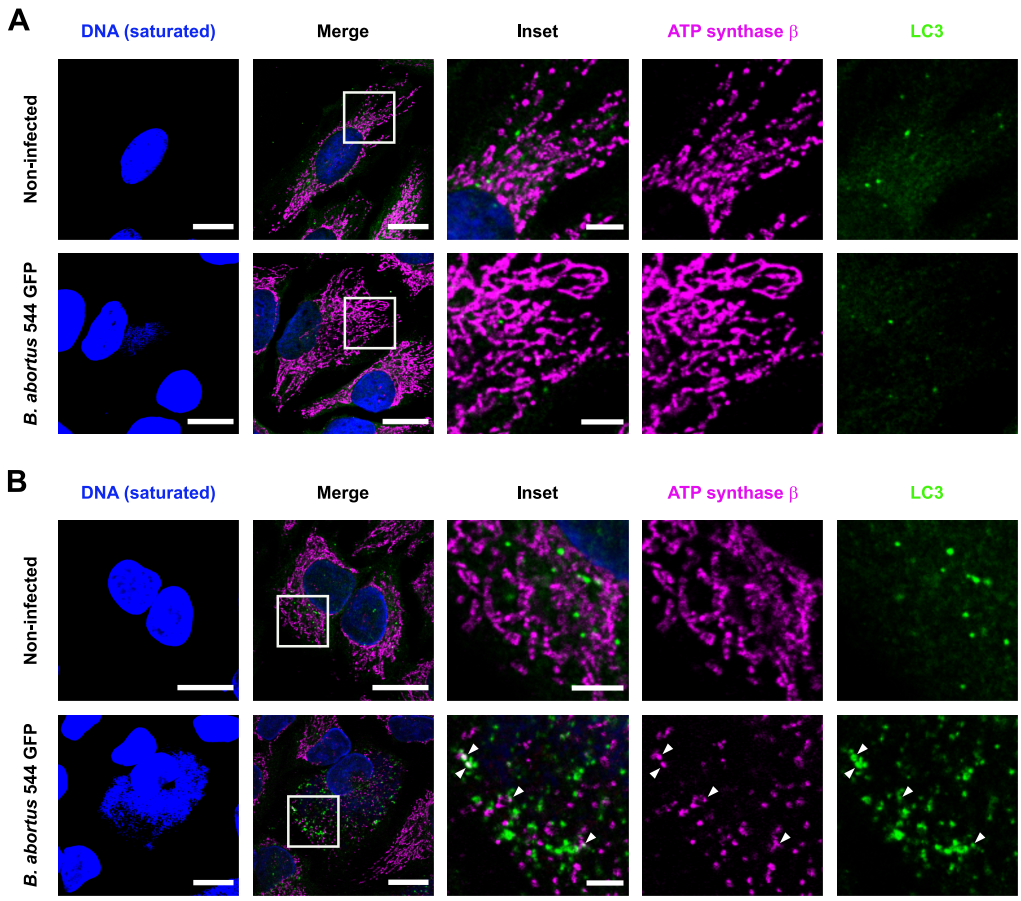
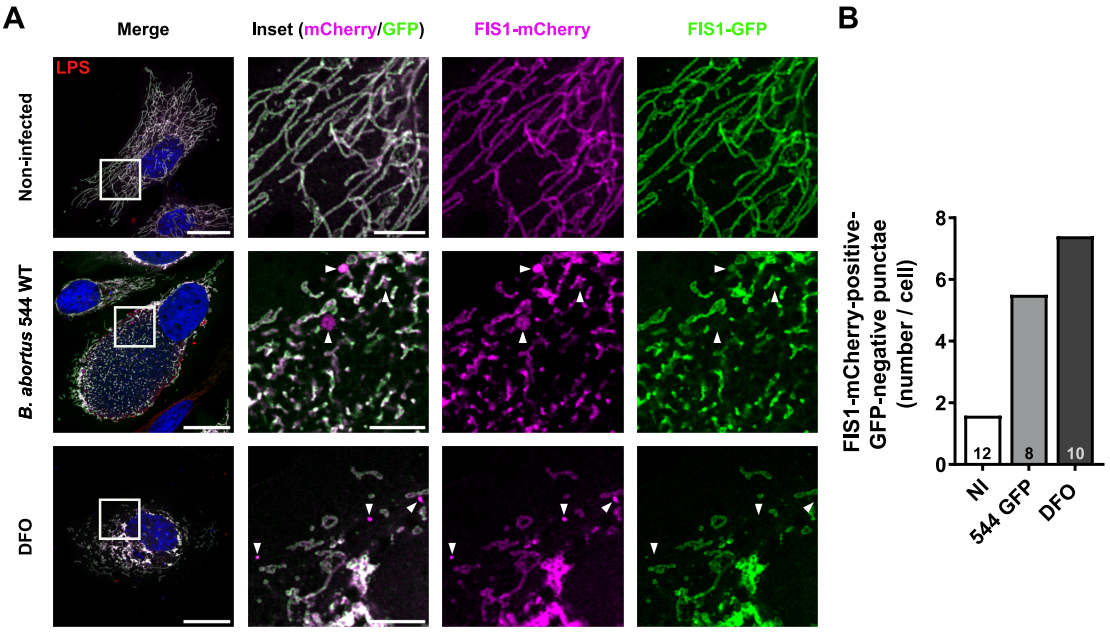



**Appendix Figure S1. Kinetics of mitophagy triggered by *B. abortus* in HeLa cells at 24 and 72 h pi.**

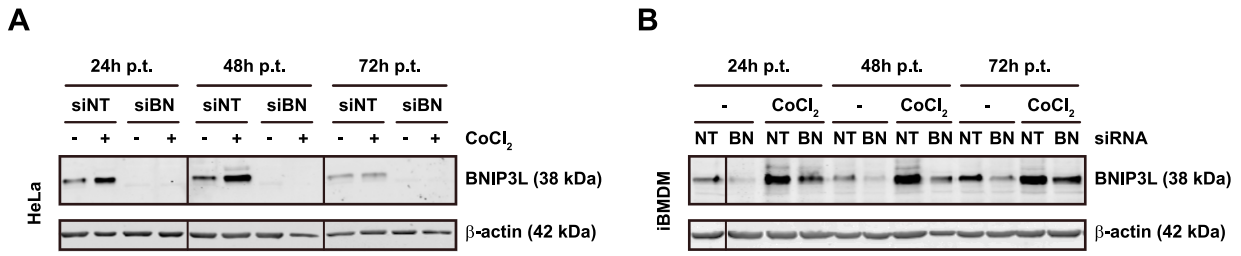
A., B. Representative confocal micrographs of HeLa cells infected or not with *B. abortus* 544 GFP for 24 h (A.) and 72 h (B.), then fixed and immunostained for the β-subunit of the ATP synthase (Alexa Fluor 633 – Magenta) and LC3 (Alexa Fluor 568 – Green). DNA was stained with Hoechst 33258 (Blue). The Hoechst intensity was intentionally shown with overexposed signals to visualise bacterial DNA and confirm the infected cells status. Scale bars: 20 µm. Inset scale bars: 5 µm.




**Appendix Figure S2. The magnitude of mitophagy induced by *B. abortus* is similar to the mitophagy induced by DFO.**

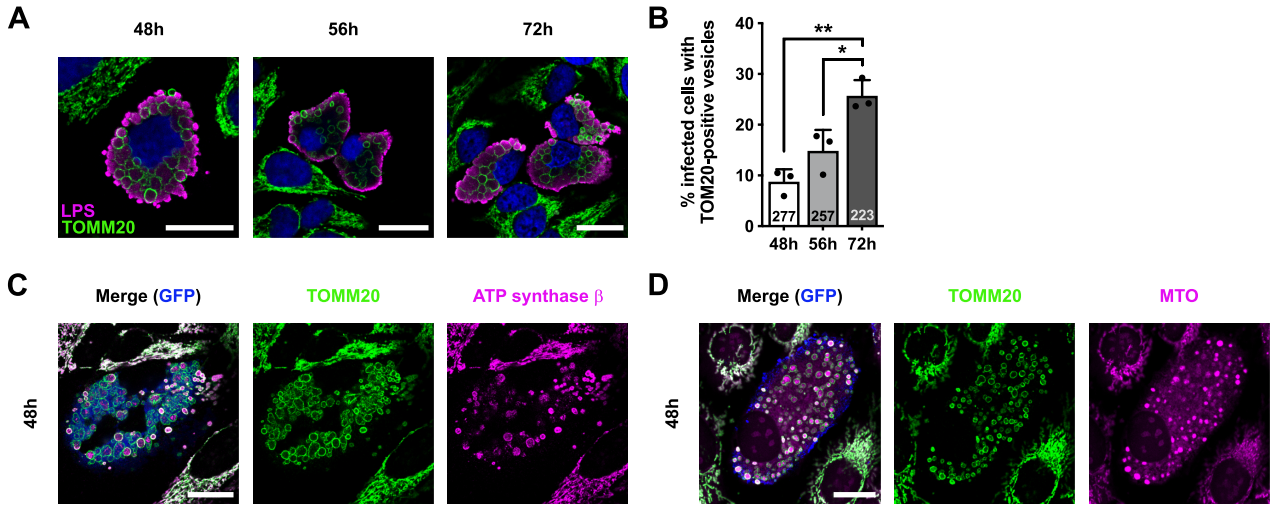
A. Representative confocal micrographs of HeLa cells transfected with a FIS1-GFP(Green)-mCherry(Magenta) expression construct, infected or not with *B. abortus* 544 for 48 h, then fixed and immunostained for *B. abortus* LPS (Alexa Fluor 633 – Red). A treatment of DFO (150 μM) for 48 h was used as a positive control. DNA was stained with Hoechst 33258 (Blue). Arrows indicate FIS1-mCherry-positive-GFP-negative punctae. Scale bars: 20 μm. Inset scale bars: 5 μm.

B. Quantification of the number of FIS1-mCherry-positive-GFP-negative punctae per HeLa cell in the indicated conditions from micrographs shown in (A). Data are presented as means from n=1 experiment (the numbers indicated in the columns represent the number of cells analysed per condition).




**Appendix Figure S3. BNIP3L depletion efficiency in HeLa cells and iBMDM using a siRNA SMARTpool approach.**

Western-blot analysis of BNIP3L abundance in HeLa cells (A) and iBMDM (B) transfected with a non-targeting siRNA pool (siNT – 40 nM) or a BNIP3L siRNA SMARTpool (siBNIP3L – 40 nM) for 24 h, then left for the indicated times post-transfection (p.t.) and treated (+) or not (-) with 100 µM $CoCl_2$ for 16 h before analysis. The abundance of β-actin was used as a loading control. Black bars indicate a crop in the membrane.




**Appendix Figure S4. *B. abortus* infection induces the swelling of mitochondria in a fraction of HeLa cells at 72 h pi.**

A. Representative confocal micrographs of HeLa cells infected or not with *B. abortus* 544 for the indicated times, then fixed and immunostained for TOMM20 (Alexa Fluor 488 – Green) and *B. abortus* LPS (Alexa Fluor 568 – Magenta). DNA was stained with Hoechst 33258 (Blue). Scale bars: 20 µm.

B. Quantification of the percentage of infected HeLa cells displaying TOMM20-positive enlarged vesicles at the indicated times, from micrographs shown in (A). Data are presented as means ± SD from n=3 (biological replicates) independent experiments (the numbers indicated in the columns represent the number of cells analysed per condition); Statistical analyses were performed using a one-way ANOVA followed by a Tukey's multiple comparisons test; *: $p$ <0.05; **: $p$ <0.01.

C. Representative confocal micrographs of HeLa cells infected or not with *B. abortus* 544 GFP (Blue) for 48 h, then fixed and immunostained for TOMM20 (Alexa Fluor 647 – Green) and the β-subunit of the ATP synthase (Alexa Fluor 568 – Magenta). Scale bars: 20 µm.

D. Representative confocal micrographs of HeLa cells infected or not with *B. abortus* 544 GFP (Blue) for 48 h, stained with 100 nM of MitoTracker™ Orange (MTO) fluorescent probe (Magenta) for 30 min before analysis, then fixed and immunostained for TOMM20 (Alexa Fluor 647 – Green). Scale bars: 20 µm.