Expanded View Figures

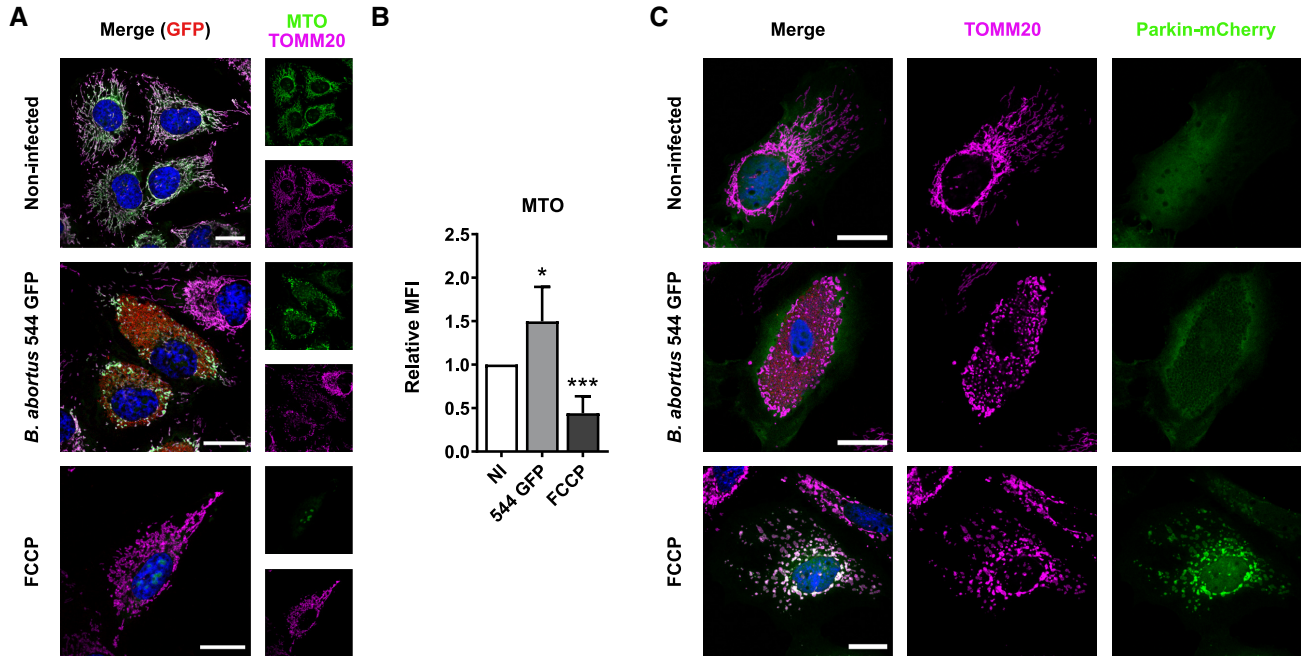

Figure EV1. ***B. abortus triggers Parkin-independent mitophagy.***

A Representative confocal micrographs of HeLa cells infected or not with *B. abortus* 544 GFP (red) for 48 h, stained with 100 nM of MTO fluorescent probe (green) for 30 min before analysis, then fixed and immunostained for TOMM20 (Alexa Fluor 633—Magenta). DNA was stained with Hoechst 33258 (blue). HeLa cells treated with 20 μM FCCP for 30 min were used as a positive control. Scale bars: 20 μm.

B Relative median fluorescence intensity (MFI) of the MTO fluorescent probe of HeLa cells infected or not (NI) with *B. abortus* 544 GFP for 48 h as measured by flow cytometry. HeLa cells treated with 20 μM FCCP for 30 min were used as a positive control. Data are presented as means ± SD from $n = 5$ (biological replicates) independent experiments (9,718 cells analysed in total per condition). Statistical analyses were performed using a one-way ANOVA followed by a Tukey's multiple comparisons test; asterisks indicate significant differences compared to the control (NI); *$P < 0.05$; ***$P < 0.001$.

C Representative confocal micrographs of HeLa cells transfected with a Parkin-mCherry (green) expression construct, infected or not with *B. abortus* 544 GFP (red) for 48 h, then fixed and immunostained for TOMM20 (Alexa Fluor 647—magenta). DNA was stained with Hoechst 33258 (blue). HeLa cells treated with FCCP (20 μM for 30 min) were used as a positive control. Scale bars: 20 μm.

Source data are available online for this figure.

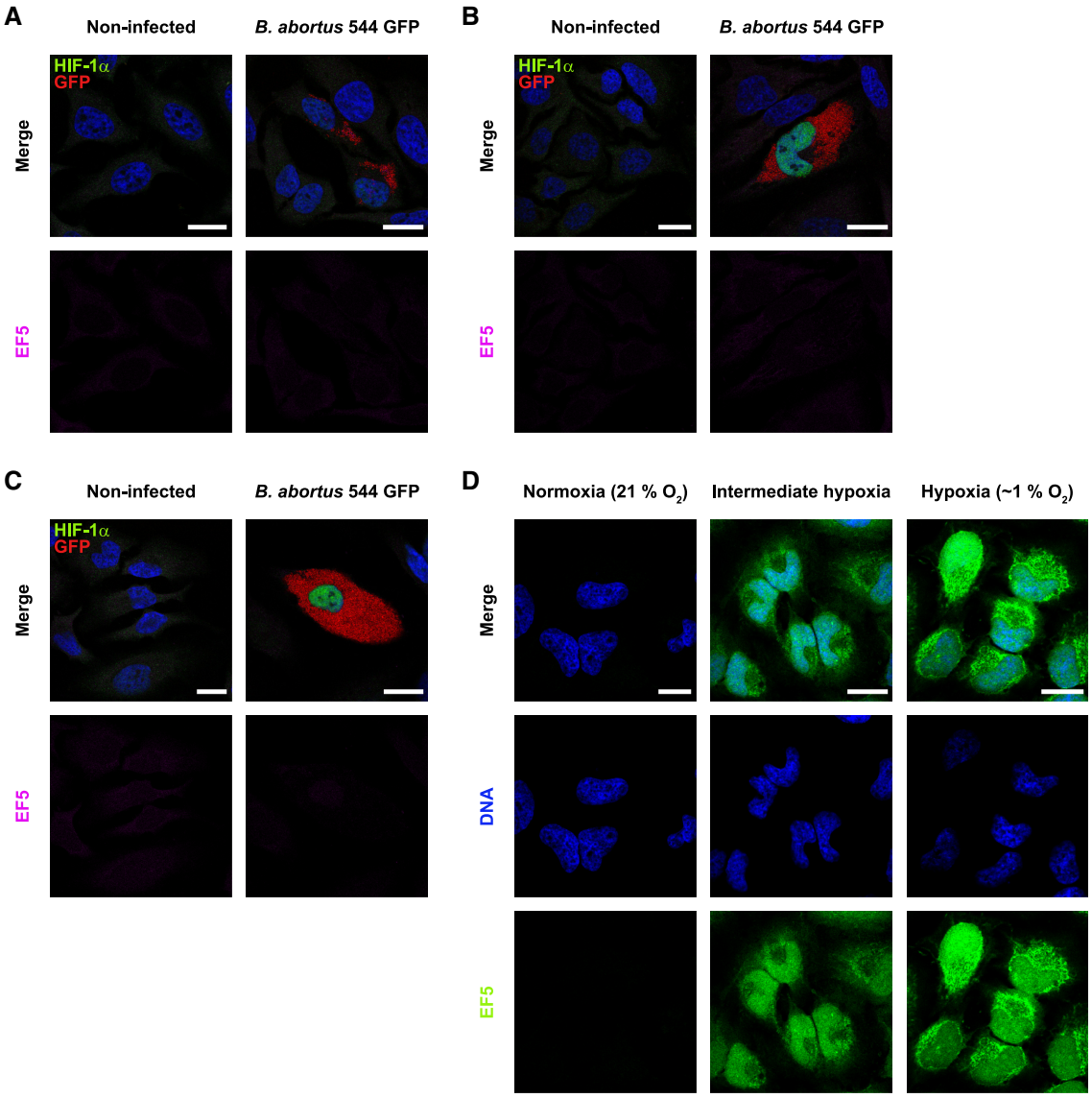

Figure EV2. *B. abortus induces HIF-1α stabilisation in a hypoxia-independent manner.*

A–C Representative confocal micrographs of HeLa cells infected or not with *B. abortus* 544 GFP (red) for 24 h (A), 48 h (B) and 72 h (C), treated with 150 µM of the EF5 compound for 3 h before analysis, then fixed and immunostained for EF5 (Anti-EF5 Cy5 conjugate—magenta) and HIF-1α (Alexa 568—green). DNA was stained with Hoechst 33258 (blue). Scale bars: 20 µm. Figure panels EV2A–C, reuse the same experiment as described in Fig 3A.

D Representative confocal micrographs of HeLa cells treated with 150 µM of the EF5 compound, exposed to normoxia (21% O_2), hypoxia (1% O_2) or an intermediate hypoxia (between 21% and 1% O_2) for 3 h, then fixed and immunostained for EF5 (anti-EF5 Cy5 conjugate—green). DNA was stained with Hoechst 33258 (blue). Scale bars: 20 µm.

Source data are available online for this figure.

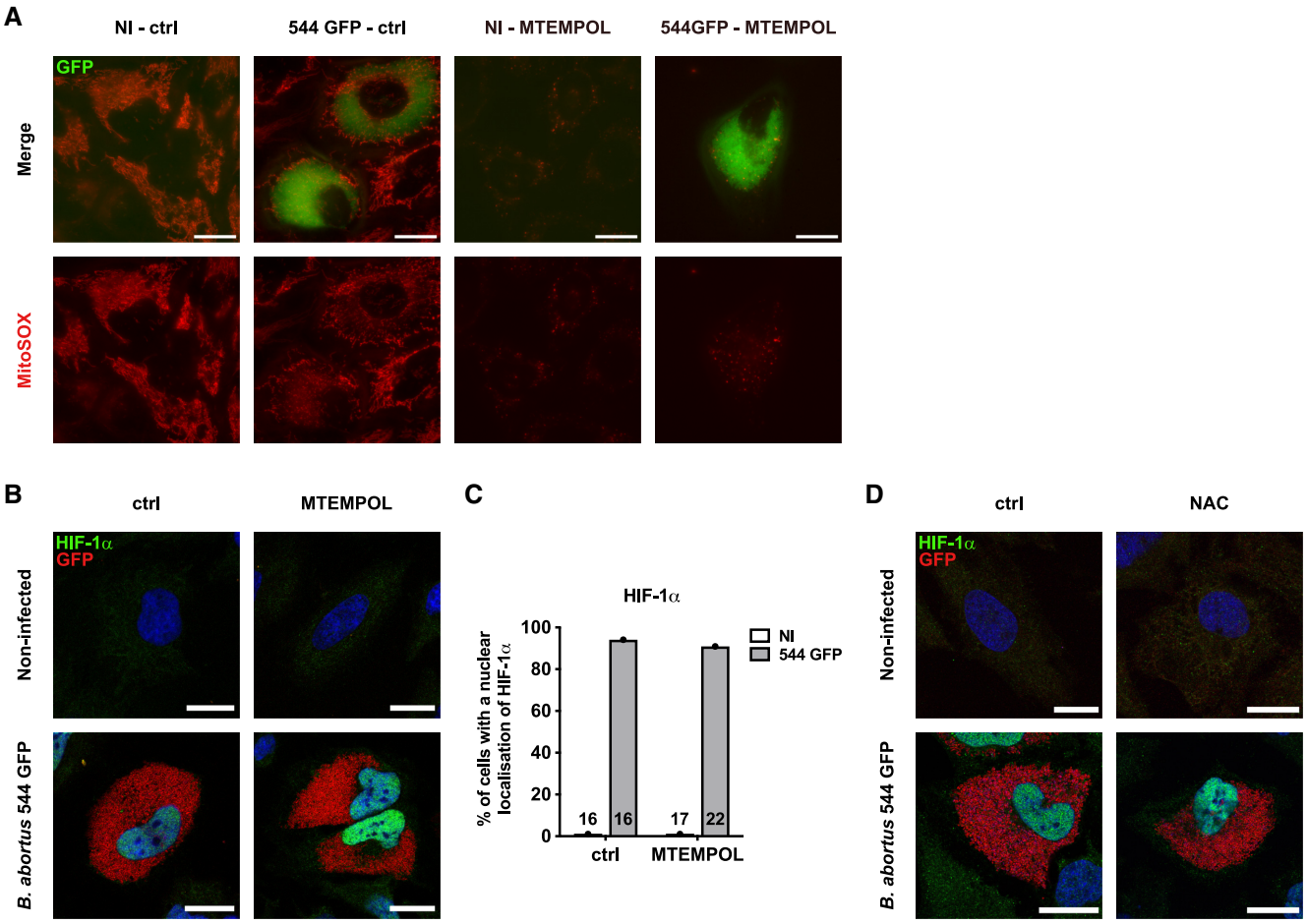

Figure EV3. ***B. abortus*-induced HIF-1α stabilisation is not mediated by mitochondrial ROS in HeLa cells.**

A Representative wide-field micrographs of HeLa cells infected or not (NI) with *B. abortus* 544 GFP (green) for 48 h, treated or not (ctrl) with 10 μM MTEMPOL for 24 h before analysis, and then stained with 0.5 μM MitoSOX™ fluorescent probe (red) for 30 min. Samples were observed under live-imaging conditions with the Nikon Eclipse Ti_2-inverted epifluorescence microscope. Scale bars: 20 μm.

B Representative confocal micrographs of HeLa cells infected or not (NI) with *B. abortus* 544 GFP (red) for 48 h, treated or not (ctrl) with 10 μM MTEMPOL for 24 h before analysis, and then fixed and immunostained for HIF-1α (Alexa 568—green). DNA was stained with Hoechst 33258 (blue). Scale bars: 20 μm.

C Quantification of the percentages of cells positive for a nuclear localisation of HIF-1α from HeLa cells infected or not (NI) with *B. abortus* 544 GFP and treated or not (ctrl) with 10 μM MTEMPOL for 24 h before analysis from micrographs shown in (B). Data are presented as means from $n = 1$ experiment (the numbers indicated in the columns represent the number of cells analysed per condition).

D Representative confocal micrographs of HeLa cells infected or not (NI) with *B. abortus* 544 GFP (red) for 48 h, treated or not (ctrl) with 5 mM NAC for 24 h before analysis, and then fixed and immunostained for HIF-1α (Alexa 568—green). DNA was stained with Hoechst 33258 (blue). Scale bars: 20 μm.

Source data are available online for this figure.

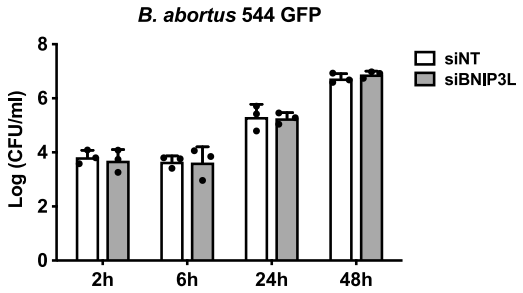

Figure EV4. BNIP3L depletion does not impair *B. abortus* intracellular replication in HeLa cells.

CFU assay expressing Log (CFU/ml) from HeLa cells transfected with a non-targeting siRNA pool (siNT—40 nM) or a BNIP3L siRNA SMARTpool (siBNIP3L—40 nM), then infected with B. abortus 544 GFP for the indicated times. Data are presented as means ± SD from n = 3 (biological replicates) independent experiments; Statistical analyses were performed using a two-way ANOVA followed by a Šidàk's multiple comparisons test; no significant differences were found.

Source data are available online for this figure.

Figure EV5. Neither BNIP3L depletion nor iron supplementation alters mBCV occurrence in HeLa cells.

A STED micrographs of a HeLa cell infected with *B. abortus* 544 GFP (magenta) for 72 h, then fixed and immunostained for TOMM20 (Abberior® STAR 635—green). The presented cell displays a rare but spectacular event of enlarged mBCVs with colonisation of the bacteria. Scale bars: 5 μm.

B Representative confocal micrographs of HeLa cells infected with *B. abortus* 544 GFP (magenta) and transfected with a non-targeting siRNA pool (siNT—40 nM) or a BNIP3L siRNA SMARTpool (siBNIP3L—40 nM) for 72 h, then fixed and immunostained for TOMM20 (Alexa Fluor 647—green). DNA was stained with Hoechst 33258 (blue). Arrows indicate when *B. abortus* was found inside a mitochondrion (mBCVs). Scale bars: 20 μm. Inset scale bars: 5 μm.

C Quantification of the number of TOMM20-positive BCVs (mBCVs) per infected HeLa cells, from micrographs shown in (B). 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 an unpaired two-tailed Student's *t*-test; ns: not significant ($P = 0.6742$).

D Representative confocal micrographs of HeLa cells infected with *B. abortus* 544 GFP (magenta), treated or not (ctrl) with $FeCl_2$ (500 μM) for 72 h, then fixed and immunostained for TOMM20 (Alexa Fluor 647—green). DNA was stained with Hoechst 33258 (blue). Arrows indicate when *B. abortus* was found inside a mitochondrion (mBCVs). Scale bars: 20 μm. Inset scale bars: 5 μm.

E Quantification of the number of TOMM20-positive BCVs (mBCVs) per infected HeLa cells, from micrographs shown in (D). 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 an unpaired two-tailed Student's *t*-test; ns, not significant ($P = 0.4724$).

F Representative confocal micrographs of HeLa cells infected with *B. abortus* 544 WT for 72 h, then fixed and immunostained for TOMM20 (Alexa Fluor 633—magenta) and LC3 (Alexa Fluor 568—green). DNA (from the HeLa nucleus and *B. abortus*) was stained with Hoechst 33258 (red). Arrows indicate when *B. abortus* was found inside a mitochondrion (mBCVs). Scale bars: 20 μm. Inset scale bars: 5 μm.

Source data are available online for this figure.

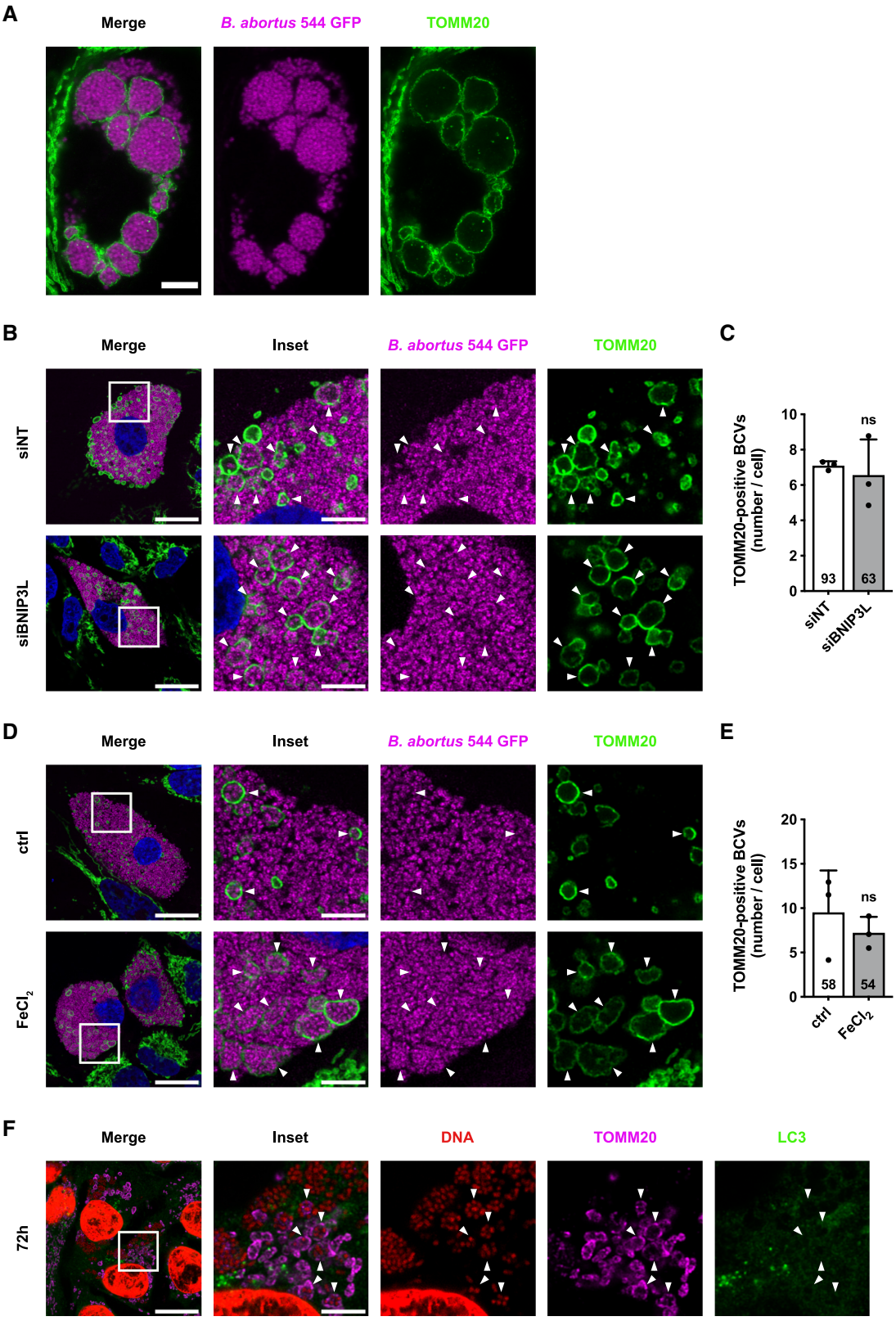

Figure EV5.