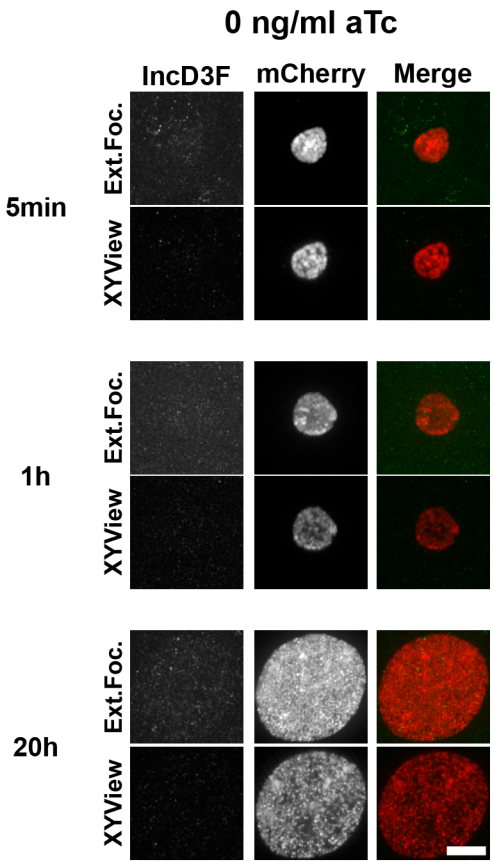

Figure S1: The IncD-3xFLAG construct is not expressed in the absence of aTc
Confocal micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3F strain. The cells were infected for 24h to allow for inclusion formation before incubation in the absence of aTc for the indicated time. The cells were fixed, immuno-stained with anti-FLAG antibodies and imaged using a confocal microscope. The same imaging settings were used for all conditions and the images were not manipulated after acquisition so that the intensity of the signals on the micrographs truly reflects the expression level of the IncD-3xFLAG construct. Left panels: IncD-3xFLAG, green. Middle panels: C. trachomatis, mCherry, red. The merge is shown on the right. The top and bottom panels respectively correspond to the extended focus view combining all the confocal planes (Ext.Foc.) and a single plane crossing the middle of the inclusion (XYView). Scale bar: 10µm.

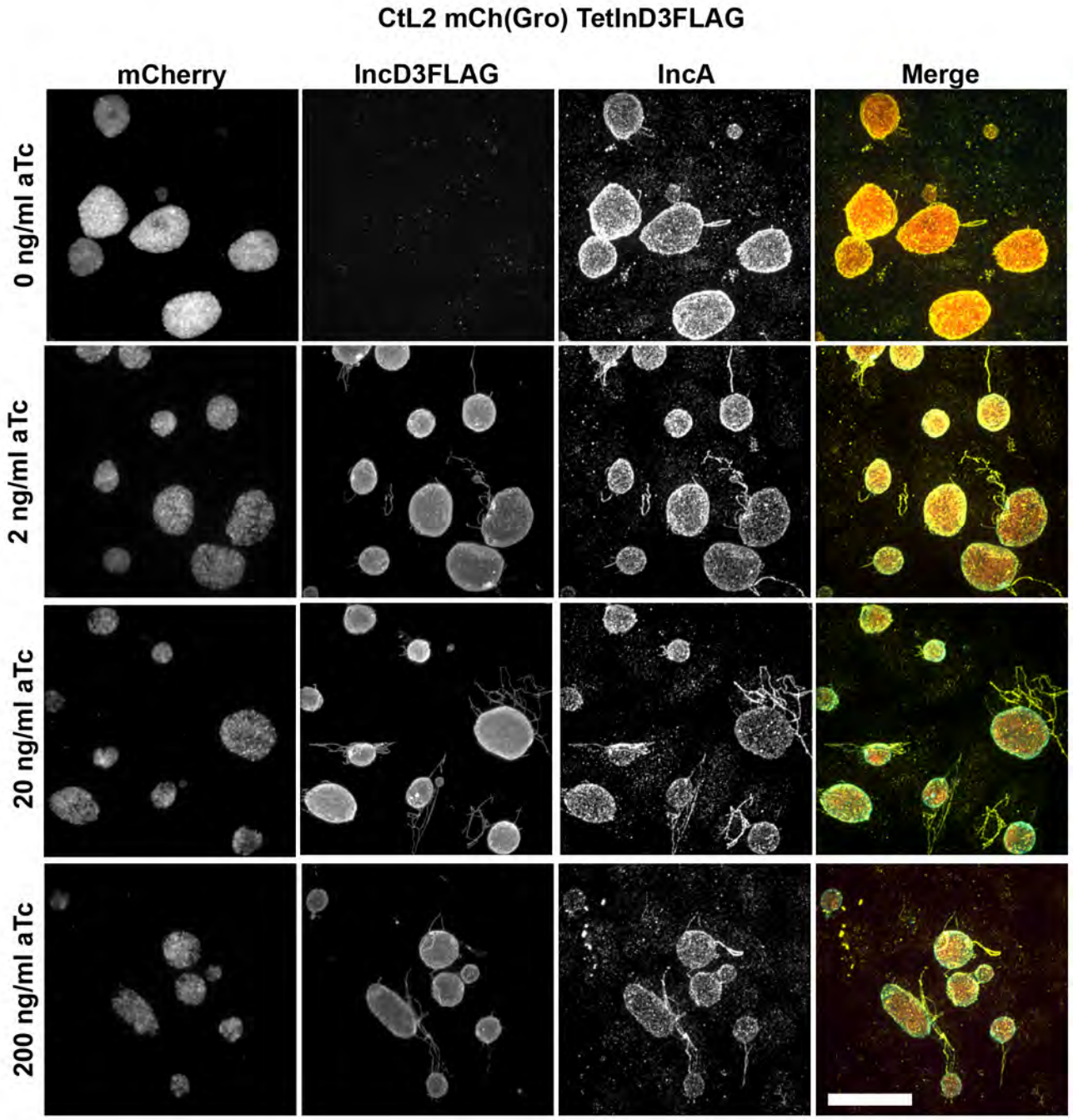

Figure S2: IncD-3xFLAG induction leads to the formation of IncA/IncD positive fibers
Confocal fluorescence micrographs of HeLa cells infected with the CtL2 mCh(Gro) TetIncD3F strain (left panels, mCherry). One hour post infection, the infected cells were incubated in the absence (top panel, 0 ng/ml aTc) or in the presence of 2, 20, 200 ng/ml of aTc. The infected cells were fixed 23h post aTc induction, co-immunostained using anti-FLAG (IncD3FLAG, blue) and anti-IncA antibodies (IncA, yellow). Extended focus view, combining the confocal planes spanning an entire inclusion, are shown. The merge is shown on the right. Scale bar: 25µm.

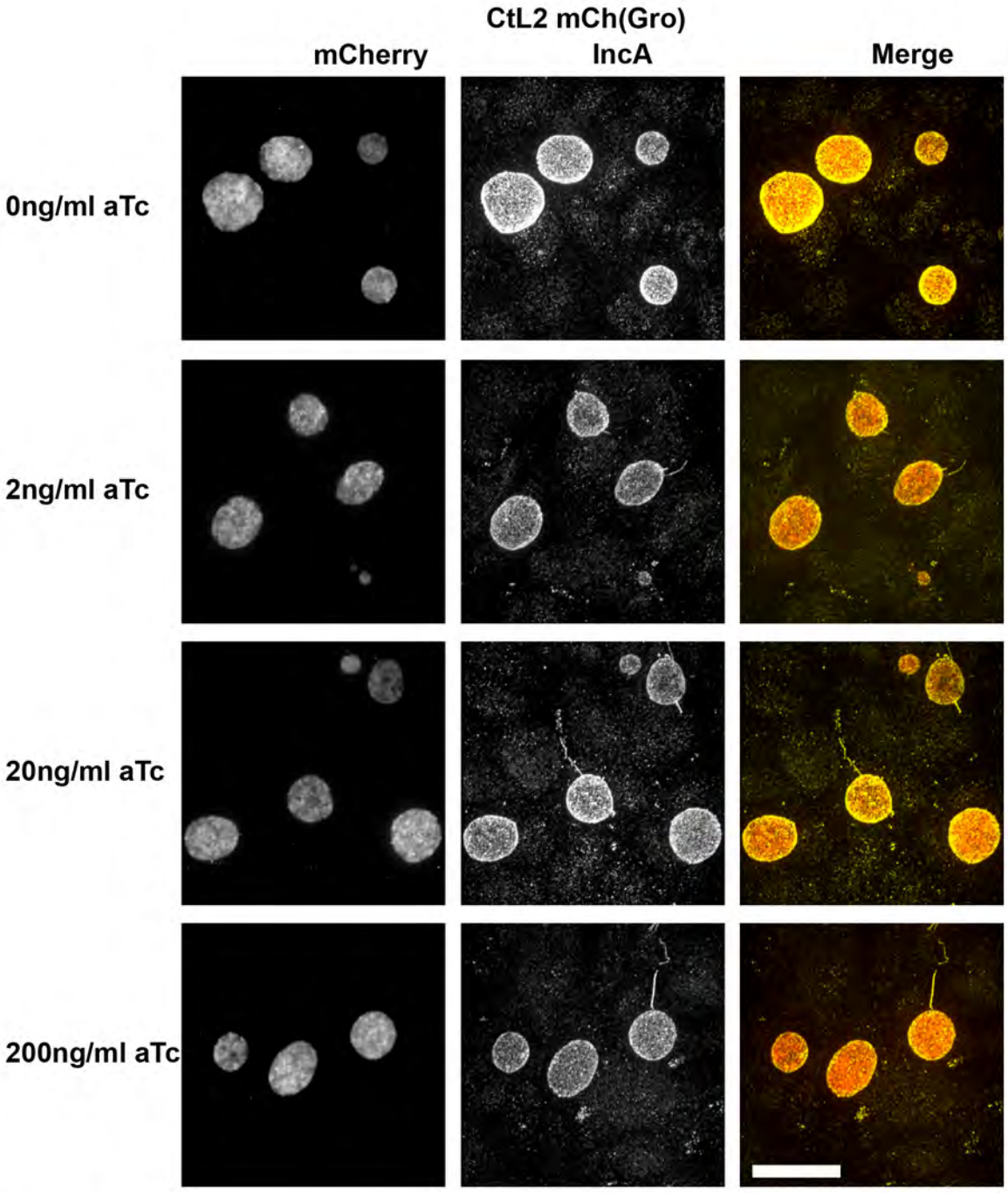

Figure S3: aTc does not induce the formation of IncA positive fibers
Confocal fluorescence micrographs of HeLa cells infected with the CtL2 mCh(Gro) strain (left panels, mCherry, red). One hour post infection, the infected cells were incubated in the absence or in the presence of 2, 20 or 200 ng/ml aTc. The infected cells were fixed 23h post aTc induction, immunostained using anti-IncA antibodies (IncA, yellow). The merge is shown on the right. Scale bar: 25µm.

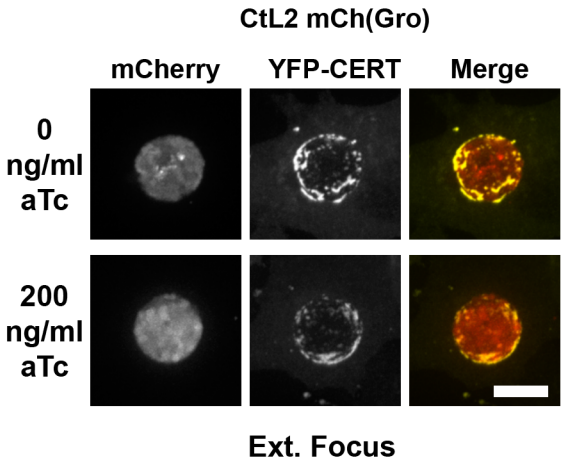

Figure S4: aTc does not affect CERT localization to the inclusion
Confocal fluorescence micrograph of inclusions of the CtL2 mCh(Gro) control strain (mCherry, red) in cells expressing a wild type YFP-CERT construct (YFP-CERT, yellow). One hour post infection, the infected cells were incubated in the absence or in the presence of the indicated aTc concentration (200 ng/ml). The infected cells were fixed 23h post aTc induction and imaged using a confocal microscope. The extended focus view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar: 10µm.

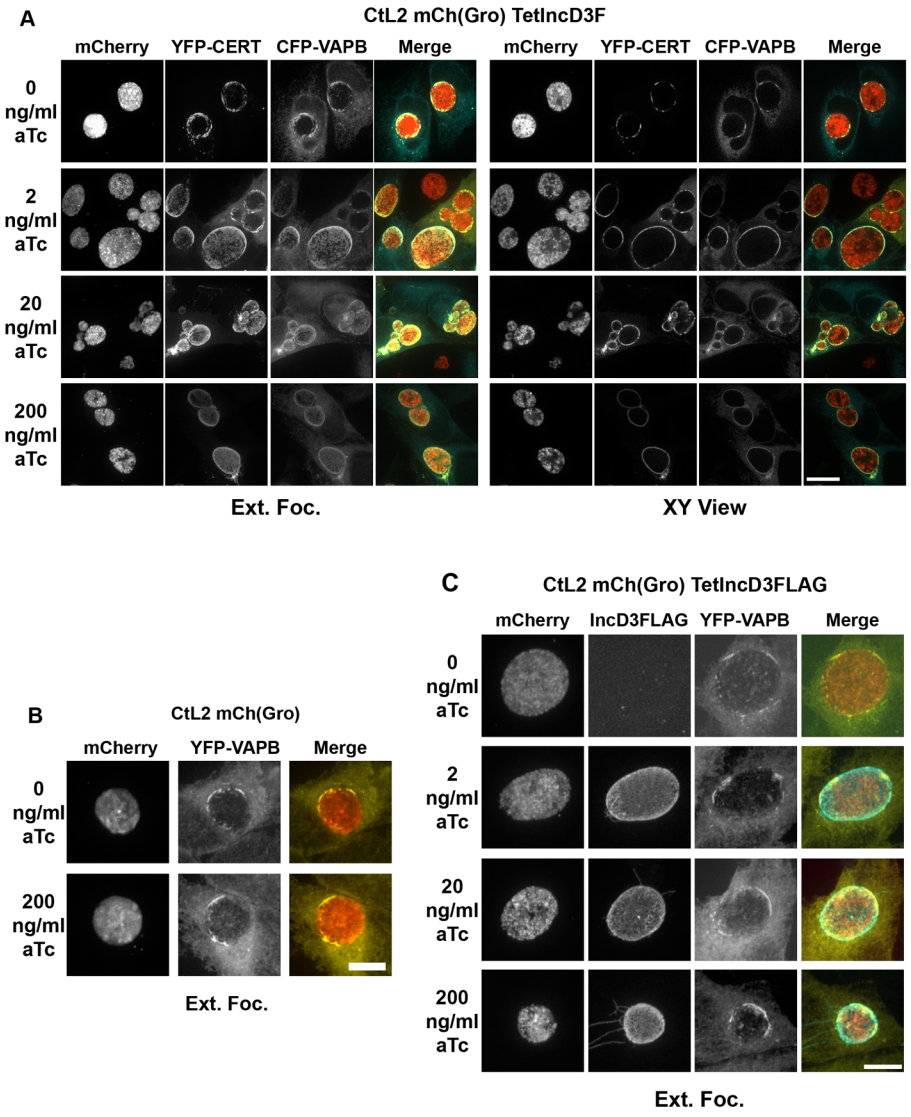

Figure S5: VAPB association with the inclusion is IncD and CERT-dependent
(A) Confocal fluorescence micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3xFLAG (mCherry, red) strain in cells co-expressing a CFP-VAPB (blue) construct and a wild type YFP-CERT (yellow) construct. One hour post infection the infected cells were incubated in the absence (0 ng/ml aTc) or in the presence of 2-20-200 ng/ml aTc. The infected cells were fixed 23h post aTc induction and imaged using a confocal microscope. The left and right panels respectively correspond to the extended focus view combining all the confocal planes (Ext.Foc.) and a single plane crossing the middle of the inclusion (XYView). The merge is shown on the right. Scale bar: 20µm. (B) Confocal fluorescence micrograph of inclusion of the CtL2 mCh(Gro) control strain (mCherry, red) in cells expressing a YFP-VAPB construct (YFP-VAPB, yellow). One hour post infectio n, the infected cells were incubated in the absence or in the presence of the indicated aTc concentration (200 ng/ml). The infected cells were fixed 23h post aTc induction and imaged using a confocal microscope. The extended focus view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar: 10µm. (C) Confocal fluorescence micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3F strain (mCherry, red) in HeLa cells expressing a YFP-VAPB construct (YFP-VAPB, yellow). One hour post infection, the infected cells were incubated in the absence (0 ng/ml aTc) or in the presence of the indicated aTc concentration (2-20-200 ng/ml aTc). The infected cells were fixed 23h post aTc induction, co-immunostained using anti-FLAG (IncD3xFLAG, blue) and imaged using a confocal microscope. The extended focus view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar: 10µm.

Table S1: Primers used in this study.

PRIMER NAME	PRIMER SEQUENCE
GroESLProm Age 5	ACCACCGGTatttttaaaaatagcagttgatcatgcc
GroESLProm Age 3	ACCACCGGTagaaaaggatggtcgtaagcactag
GroESL mCherry START 5	gaatataaaaatacgaggagcttaaacATGGTGAGCAAGGGCGAGGAGG
GroESL mCherry START 3	CCTCCTCGCCCTTGCTCACCATgtttaagctcctcgtatttttatattc
GroESL mCherry STOP 5	GGACGAGCTGTACAAGTAGttcctctaatgggaacaaatag
GroESL mCherry STOP 3	ctatttgttcccattagaggaaCTACTTGTACAGCTCGTCC
IncD 3xFLAG 3	TTAATCGTCATCCTTGTAATCGATGTCATGATCTTTATAATCACCG TCATGGTCTTTGTAGTCcatGCTCGCCCCTTTTTTACTCACCG
D FLAG STOP 3	cgcgaatcacatgtcatccTTAATCGTCATCCTTGTAATCG
D FLAG STOP 5	CGATTACAAGGATGACGATTAAggatgacatgtgattcgcg
IncD Term NotI	GCGGGCGGCCGCgtcttaggagcttttgcaatgc
TetAP-IncD3F 5	cagtgatagagaaaagtgaaATGACGAAGGTTTATGCGCATAGC
TetAP-IncD3F 3	GCTATGCGCATAAACCTTCGTCATttcacttttctctatcactg
TetR STOP 5 Kpn	GGTGGTACCTTAAGACCCACTTTCACATTTAAG
IncDTerm 3 Not	GCGGGCGGCCGCgtcttaggagcttttgcaatgc

Supplementary Figure Legends

Supplementary Figure S1: The IncD-3xFLAG construct is not expressed in the absence of aTc

Confocal micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3F strain. The cells were infected for 24h to allow for inclusion formation before incubation in the absence of aTc for the indicated time. The cells were fixed, immuno-stained with anti-FLAG antibodies and imaged using a confocal microscope. The same imaging settings were used for all conditions and the images were not manipulated after acquisition so that the intensity of the signals on the micrographs truly reflects the expression level of the IncD-3xFLAG construct. Left panels: IncD-3xFLAG, green. Middle panels: *C. trachomatis*, mCherry, red. The merge is shown on the right. The top and bottom panels respectively correspond to the extended focus view combining all the confocal planes (Ext.Foc.) and a single plane crossing the middle of the inclusion (XYView). Scale bar: 10µm.

Supplementary Figure S2: IncD-3xFLAG induction leads to the formation of IncA/IncD positive fibers

Confocal fluorescence micrographs of HeLa cells infected with the CtL2 mCh(Gro) TetIncD3F strain (left panels, mCherry). One hour post infection, the infected cells were incubated in the absence (top panel, 0 ng/ml aTc) or in the presence of 2, 20, 200 ng/ml of aTc. The infected cells were fixed 23h post aTc induction, co-immunostained using anti-FLAG (IncD3FLAG, blue) and anti-IncA antibodies (IncA, yellow). Extended focus

view, combining the confocal planes spanning an entire inclusion, are shown. The merge is shown on the right. Scale bar: 25µm.

Supplementary Figure S3: aTc does not induce the formation of IncA positive fibers

Confocal fluorescence micrographs of HeLa cells infected with the CtL2 mCh(Gro) strain (left panels, mCherry, red). One hour post infection, the infected cells were incubated in the absence or in the presence of 2, 20 or 200 ng/ml aTc. The infected cells were fixed 23h post aTc induction, immunostained using anti-IncA antibodies (IncA, yellow). The merge is shown on the right. Scale bar: 25µm.

Supplementary Figure S4: aTc does not affect CERT localization to the inclusion

Confocal fluorescence micrograph of inclusions of the CtL2 mCh(Gro) control strain (mCherry, red) in cells expressing a wild type YFP-CERT construct (YFP-CERT, yellow). One hour post infection, the infected cells were incubated in the absence or in the presence of the indicated aTc concentration (200 ng/ml). The infected cells were fixed 23h post aTc induction and imaged using a confocal microscope. The extended focus view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar: 10µm.

Supplementary Figure S5: VAPB association with the inclusion is IncD and CERT-dependent

(A) Confocal fluorescence micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3xFLAG (mCherry, red) strain in cells co-expressing a CFP-VAPB (blue)

construct and a wild type YFP-CERT (yellow) construct. One hour post infection the infected cells were incubated in the absence (0 ng/ml aTc) or in the presence of 2-20-200 ng/ml aTc. The infected cells were fixed 23h post aTc induction and imaged using a confocal microscope. The left and right panels respectively correspond to the extended focus view combining all the confocal planes (Ext.Foc.) and a single plane crossing the middle of the inclusion (XYView). The merge is shown on the right. Scale bar: 20µm. (B) Confocal fluorescence micrograph of inclusion of the CtL2 mCh(Gro) control strain (mCherry, red) in cells expressing a YFP-VAPB construct (YFP-VAPB, yellow). One hour post infection, the infected cells were incubated in the absence or in the presence of the indicated aTc concentration (200 ng/ml). The infected cells were fixed 23h post aTc induction and imaged using a confocal microscope. The extended focus view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar: 10µm. (C) Confocal fluorescence micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3F strain (mCherry, red) in HeLa cells expressing a YFP-VAPB construct (YFP-VAPB, yellow). One hour post infection, the infected cells were incubated in the absence (0 ng/ml aTc) or in the presence of the indicated aTc concentration (2-20-200 ng/ml aTc). The infected cells were fixed 23h post aTc induction, co-immunostained using anti-FLAG (IncD3xFLAG, blue) and imaged using a confocal microscope. The extended focus view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar: 10µm.