

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.

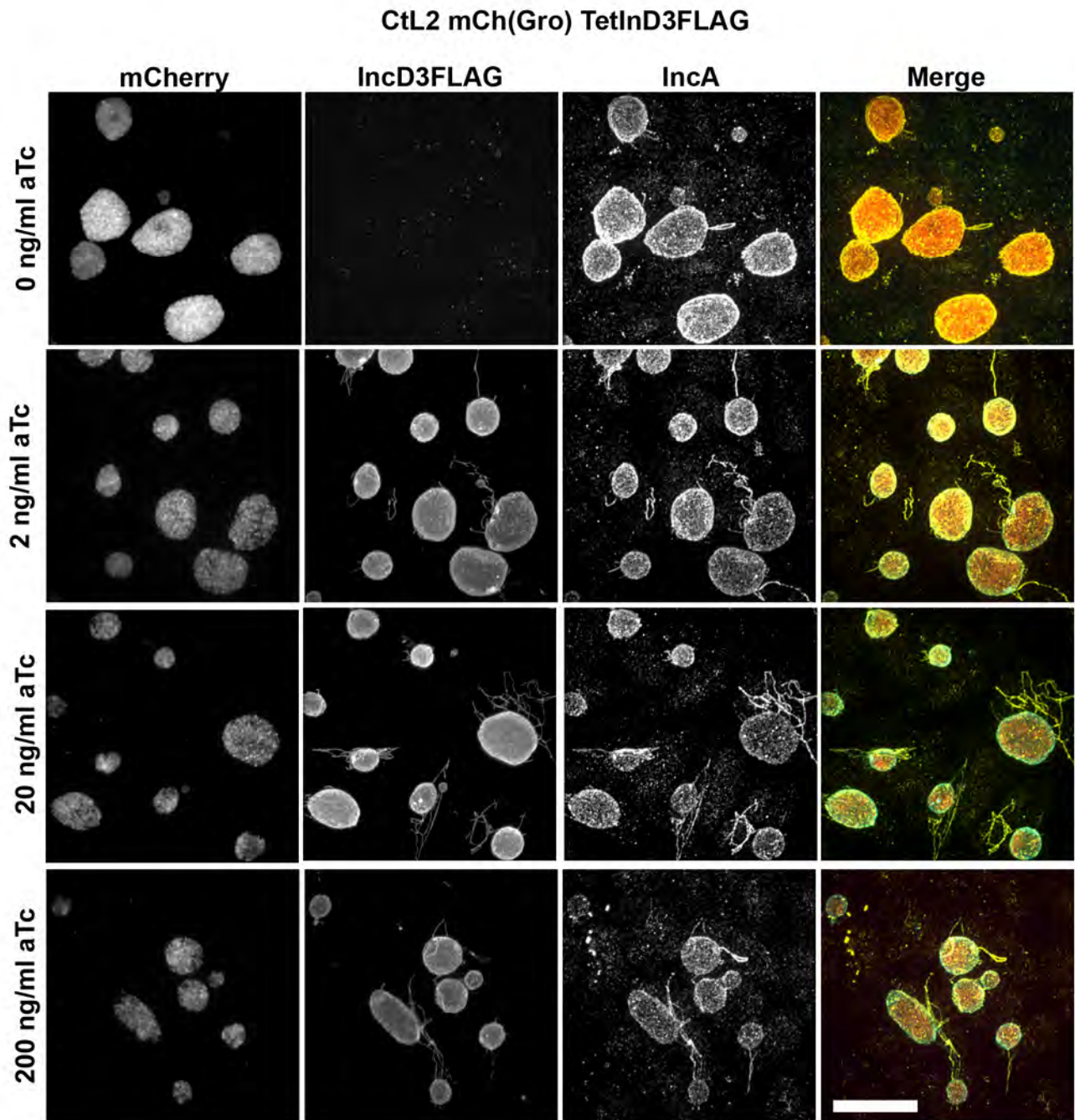


Figure S2: IncD-3xFLAG induction leads to the formation of Inca/IncD positive fibers
 Confocal fluorescence micrographs of HeLa cells infected with the CtL2 mCh(Gro) TetIncd3F strain (left panels, mCherry). One hour post infection, the infected cells were incubated in the absence (top panel, 0 ng/ml 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.

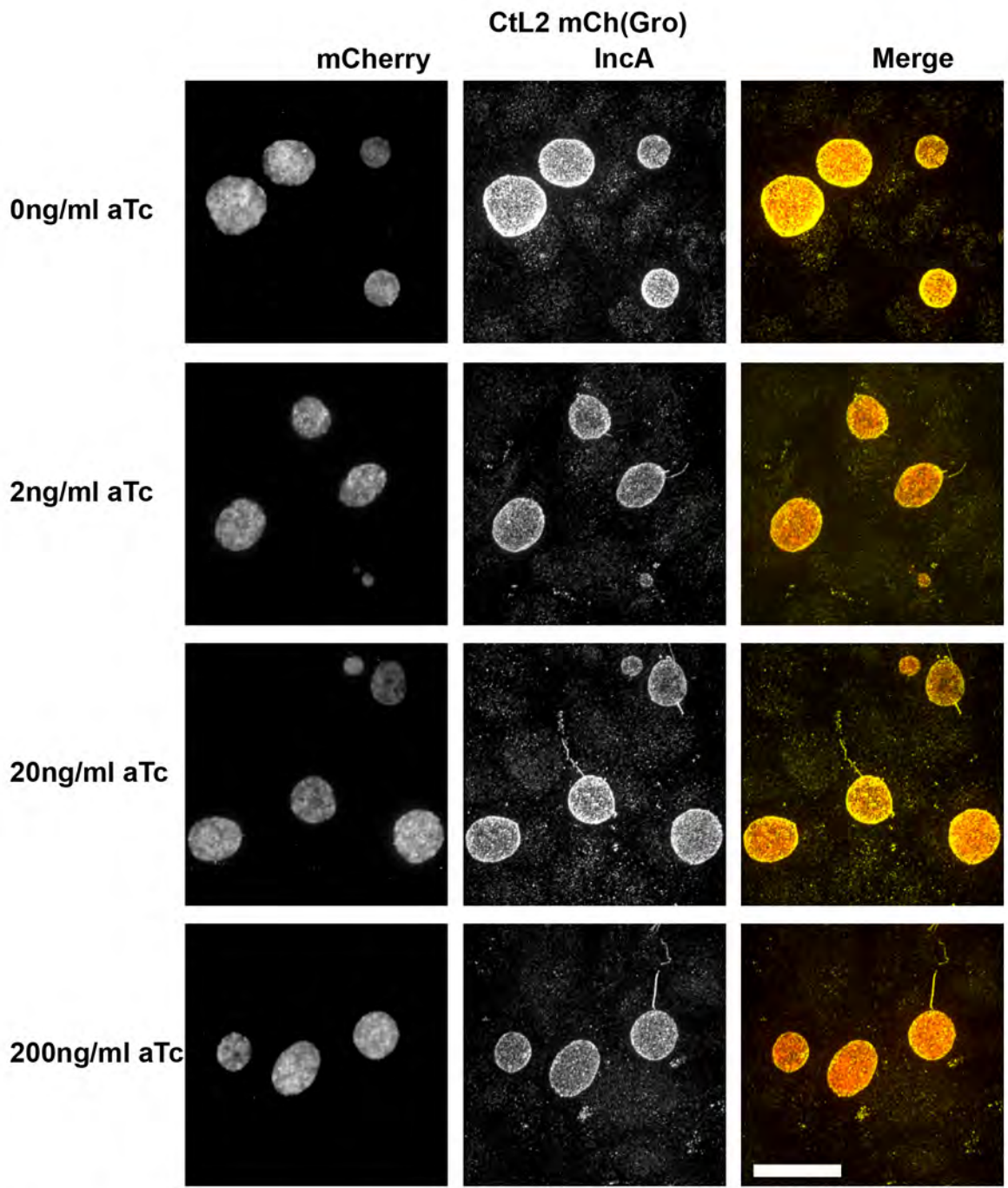


Figure S3: aTc does not induce the formation of IncA positive fibers
 Confocal fluorescence micrographs of HeLa cells infected with the CtL2 mCh(Gro) strain (left panels, mCherry, red). One hour post infection, the infected cells were incubated in the absence or in the presence of 2, 20 or 200 ng/ml 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.

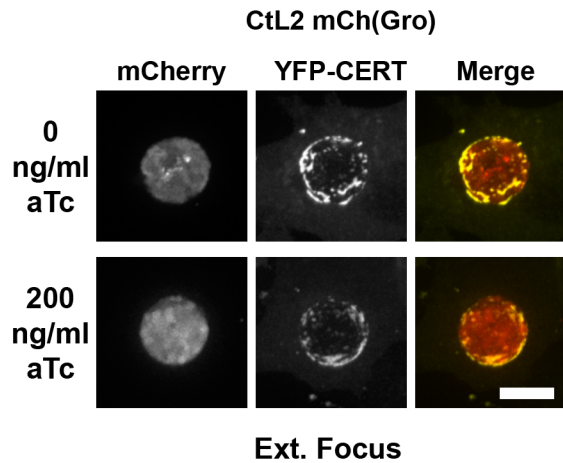


Figure S4: aTc does not affect CERT localization to the inclusion

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.

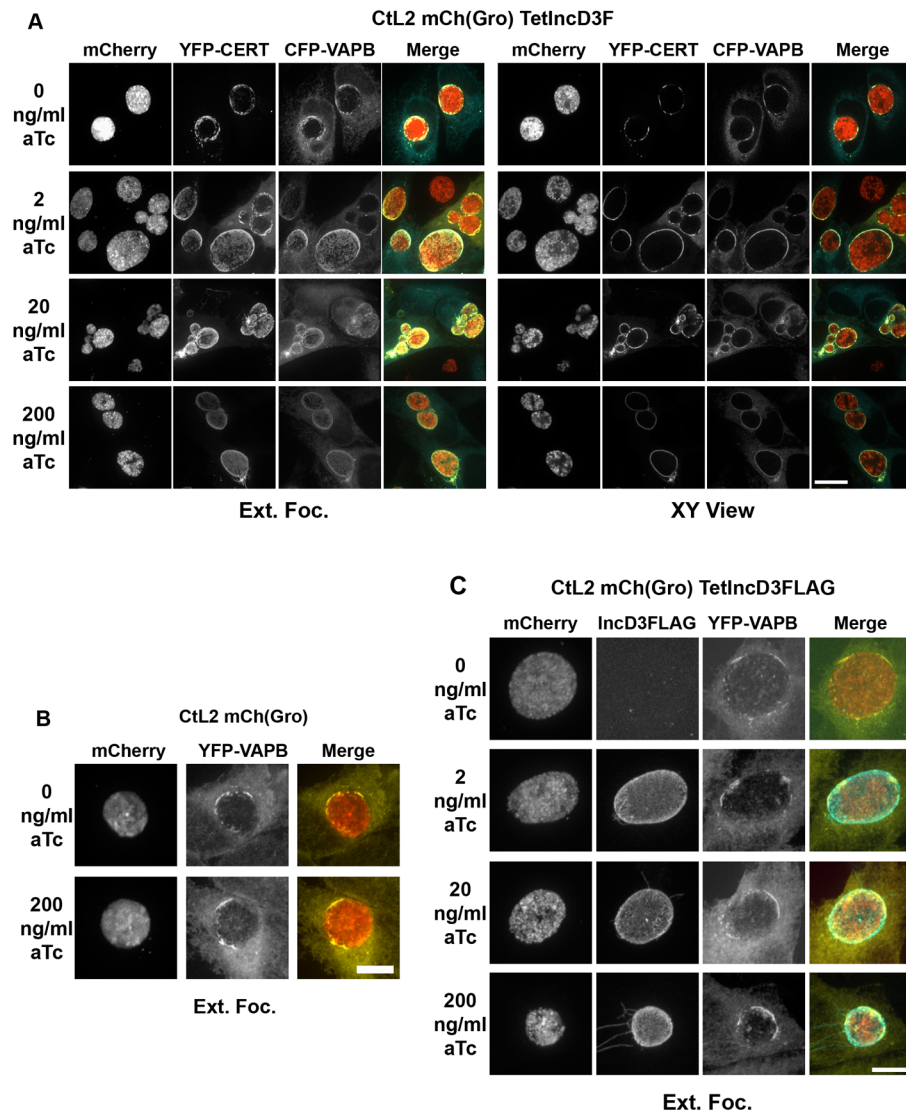


Figure S5: VAPB association with the inclusion is IncD and CERT-dependent

(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 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.

Table S1: Primers used in this study.

PRIMER NAME	PRIMER SEQUENCE
GroESLProm Age 5	ACCACCGGTatnttaaaaaatagcagttgatcatgcc
GroESLProm Age 3	ACCACCGGTagaaaaggatggctgtaagcactag
GroESL mCherry START 5	gaatataaaaatacaggagacttaaacATGGTGAGCAAGGGCGAGGAGG
GroESL mCherry START 3	CCTCCTCGCCCTTGCTCACCATgtttaagctcctcgatnttatattc
GroESL mCherry STOP 5	GGACGAGCTGTACAAGTAGttcctctaattgggaacaaatag
GroESL mCherry STOP 3	ctatttgttcccattagaggaaCTACTTGTACAGCTCGTCC
IncD 3xFLAG 3	TTAATCGTCATCCTTGTAATCGATGTCATGATCTTTATAATCACCG TCATGGTCTTTGTAGTCcatGCTCGCCCCTTTTTACTCACCG
D FLAG STOP 3	cgcgatcacatgcatccTTAATCGTCATCCTTGTAATCG
D FLAG STOP 5	CGATTACAAGGATGACGATTAAggatgacatgtgattcgcg
IncD Term NotI	GCGGGCGGCCGCgtcttaggagcttttgcaatgc
TetAP-IncD3F 5	cagtgatagagaaaagtgaaATGACGAAGGTTTATGCGCATAGC
TetAP-IncD3F 3	GCTATGCGCATAAACCTTCGTCATttcacttttctctactg
TetR STOP 5 Kpn	GGTGGTACCTTAAGACCCACTTTCACATTTAAG
IncDTerm 3 Not	GCGGGCGGCCGCgtcttaggagcttttgcaatgc

1 **Supplementary Figure Legends**

2

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

5 Confocal micrographs of inclusions of the CtL2 mCh(Gro) TetIncD3F strain. The cells
6 were infected for 24h to allow for inclusion formation before incubation in the absence of
7 aTc for the indicated time. The cells were fixed, immuno-stained with anti-FLAG
8 antibodies and imaged using a confocal microscope. The same imaging settings were
9 used for all conditions and the images were not manipulated after acquisition so that the
10 intensity of the signals on the micrographs truly reflects the expression level of the IncD-
11 3xFLAG construct. Left panels: IncD-3xFLAG, green. Middle panels: *C. trachomatis*,
12 mCherry, red. The merge is shown on the right. The top and bottom panels respectively
13 correspond to the extended focus view combining all the confocal planes (Ext.Foc.) and a
14 single plane crossing the middle of the inclusion (XYView). Scale bar: 10 μ m.

15

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

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

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

25

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

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

32

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

34 Confocal fluorescence micrograph of inclusions of the CtL2 mCh(Gro) control strain
35 (mCherry, red) in cells expressing a wild type YFP-CERT construct (YFP-CERT,
36 yellow). One hour post infection, the infected cells were incubated in the absence or in
37 the presence of the indicated aTc concentration (200 ng/ml). The infected cells were fixed
38 23h post aTc induction and imaged using a confocal microscope. The extended focus
39 view combining all the confocal planes spanning each inclusion is shown (Ext.Foc.). The
40 merge is shown on the right. Scale bar: 10 μ m.

41

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

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

46 construct and a wild type YFP-CERT (yellow) construct. One hour post infection the
47 infected cells were incubated in the absence (0 ng/ml aTc) or in the presence of 2-20-200
48 ng/ml aTc. The infected cells were fixed 23h post aTc induction and imaged using a
49 confocal microscope. The left and right panels respectively correspond to the extended
50 focus view combining all the confocal planes (Ext.Foc.) and a single plane crossing the
51 middle of the inclusion (XYView). The merge is shown on the right. Scale bar: 20 μ m.
52 (B) Confocal fluorescence micrograph of inclusion of the CtL2 mCh(Gro) control strain
53 (mCherry, red) in cells expressing a YFP-VAPB construct (YFP-VAPB, yellow). One
54 hour post infection, the infected cells were incubated in the absence or in the presence of
55 the indicated aTc concentration (200 ng/ml). The infected cells were fixed 23h post aTc
56 induction and imaged using a confocal microscope. The extended focus view combining
57 all the confocal planes spanning each inclusion is shown (Ext.Foc.). The merge is shown
58 on the right. Scale bar: 10 μ m. (C) Confocal fluorescence micrographs of inclusions of the
59 CtL2 mCh(Gro) TetIncD3F strain (mCherry, red) in HeLa cells expressing a YFP-VAPB
60 construct (YFP-VAPB, yellow). One hour post infection, the infected cells were
61 incubated in the absence (0 ng/ml aTc) or in the presence of the indicated aTc
62 concentration (2-20-200 ng/ml aTc). The infected cells were fixed 23h post aTc
63 induction, co-immunostained using anti-FLAG (IncD3xFLAG, blue) and imaged using a
64 confocal microscope. The extended focus view combining all the confocal planes
65 spanning each inclusion is shown (Ext.Foc.). The merge is shown on the right. Scale bar:
66 10 μ m.
67
68
69