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Impact of capsaicin, an active component of chili pepper, on pathogenic chlamydial growth (*Chlamydia trachomatis* and *Chlamydia pneumoniae*) in immortal human epithelial HeLa cells

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Abstract

Chlamydia trachomatis is the leading cause of sexually transmitted infections worldwide. Capsaicin, a component of chili pepper, which can stimulate actin remodeling via capsaicin receptor TRPV1 (transient receptor potential vanilloid 1) and anti-inflammatory effects via PPARγ (peroxisome proliferator-activated receptor-γ) and LXRα (liver X receptor α), is a potential candidate to control chlamydial growth in host cells. We examined whether capsaicin could inhibit *C. trachomatis* growth in immortal human epithelial HeLa cells. Inclusion forming unit and quantitative PCR assays showed that capsaicin significantly inhibited bacterial growth in cells in a dose-dependent manner, even in the presence of cycloheximide, a eukaryotic protein synthesis inhibitor. Confocal microscopic and transmission electron microscopic observations revealed an obvious decrease in bacterial numbers to inclusions bodies formed in the cells. Although capsaicin can stimulate the apoptosis of cells, no increase in cleaved PARP (poly (ADP-ribose) polymerase), an apoptotic indicator, was observed at a working concentration. All of the drugs tested (capsazepine, a TRPV1 antagonist; 5CPPSS-50, an LXRα inhibitor; and T0070907, a PPARγ inhibitor) had no effect on chlamydial inhibition in the presence of capsaicin. In addition, we also confirmed that capsaicin inhibited *Chlamydia pneumoniae* growth, indicating a phenomena not specific to *C. trachomatis*. Thus, we conclude that capsaicin can block chlamydial growth without the requirement of host cell protein synthesis, but by another, yet to be defined, mechanism.

Keywords: *Chlamydia trachomatis*; capsaicin; HeLa cells; TRPV1; PPARγ; LXRα

Introduction

Chlamydia trachomatis is an obligate intracellular bacterium with a unique developmental cycle, consisting of elementary (EB) and reticulate body (RB) forms. Differentiation from EB to RB (or re-differentiation from RB to EB) forms occurs in inclusion bodies [1]. The maturation process from EB to RB forms in infected host cells has been investigated and requires actin remodeling [2-4], lipid metabolism [5-7] and inflammatory responses [8-10], although it remains to be fully understood.

Chlamydia are the leading cause of bacterial sexually transmitted diseases worldwide, with an estimated 100 million chlamydial infections detected annually [11]. Such infections are often asymptomatic in women [12-14] and can be left untreated, resulting in serious outcomes with ductal obstruction, pelvic inflammatory disease, tubal occlusion and extrauterine pregnancy [15-17]. The prevalence of *C. trachomatis* following prophylactic countermeasures therefore needs to be investigated.

The use of natural compounds that are easily and safely ingested within a daily diet is a new approach to prophylactically overcome a variety of infectious risks. For example, chili peppers contain capsaicin (8-methyl-N-vanillyl-6-nonenamide), a phenolic compound produced by all plants [18], that has been well investigated [19-22]. Capsaicin can inhibit the growth of human pathogenic bacteria such as cell-invasive group A streptococci [20], *Vibrio cholera* [21], *Escherichia coli* O157:H7 [22] and *Salmonella enterica* serovar Typhimurium [22], indicating its potential application against a broad range of bacterial infectious diseases.

Capsaicin can also modulate cellular dynamics by actin remodeling via the capsaicin receptor TRPV1 (transient receptor potential vanilloid 1), which is a channel expressed at a subset of sensory neurons involved in pain sensation and other

non-neuronal sites in mammalian cells [18, 23], and by exerting anti-inflammatory effects via PPARγ (peroxisome proliferator-activated receptor-gamma) [18, 24] and LXRα (liver X receptor α) [18, 24]. Specifically, TRPV1 activation altered F-actin organization in an ERK1/2- and MLC2-dependent manner [23], and the inhibitory action of capsaicin on lipopolysaccharide (LPS)-induced IL-1β, IL-6 and TNF-α production occurred depending on the activation of both PPARγ and LXRα [24].

Thus, interestingly, it appears that the activity of capsaicin is similar to that of chlamydia, raising the idea that bacteria and capsaicin may compete with resulting effects on actin remodeling and inflammatory activity, and ultimately chlamydial growth inhibition. We therefore examined whether capsaicin can inhibit *C. trachomatis* growth in immortal human epithelial HeLa cells. Here, for the first time, we showed that capsaicin can inhibit chlamydial growth with minimal requirement for host responses.

Materials and methods

Drugs

Capsaicin (Tokyo Chemical Industry Co., Ltd.) was resolved in 95% ethanol at a stock concentration, 50 mg/ml. Capsazepine (Wako) (antagonist for TRPV1), 5CPPSS-50 (Wako) (inhibitor for LXRα), and T0070907 (CAY) (inhibitor for PPARγ) were resolved in DMSO at 50, 15 and 50 mM, respectively. Cycloheximide (eukaryote-specific protein synthesis inhibitor) purchased from Sigma was resolved in DMSO at 20 mg/ml. These drugs were stored at -80 °C until use.

Immortal human cell lines

Immortal human epithelial HeLa and HEp-2 cells were purchased from the American Type Culture Collection and the Riken Cell Bank (Tsukuba, Japan), respectively. Both of the cell lines were cultured at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle's Medium (Sigma) containing 10% heat-inactivated fetal calf serum and antibiotics [gentamicin (10 μg/ml) (Sigma), vancomycin (10 μg/ml) (Sigma) and amphotericin B (0.5 μg/ml) (Sigma)] [25, 26].

Bacteria and inclusion forming unit (IFU) assay

C. trachomatis strain UW-3/CX (*ompA* genotype: D) was purchased from ATCC. *Chlamydia pneumoniae* strain TW183 was kindly provided by G. Byrne, University of Tennessee (Memphis, TN, USA). *C. trachomatis* and *C. pneumoniae* were propagated in HeLa and HEp-2 cells, respectively, as described previously [25, 26]. In brief, the infected cells were harvested on day 2 (for *C. trachomatis*) or day 3 (for *C. pneumoniae*), and disrupted by freezing-thawing. After centrifugation to remove cell debris, bacteria were collected by high-speed centrifugation. Bacterial pellets were resuspended in sucrose–phosphate–glutamic acid buffer (0.2 M sucrose, 3.8 mM KH_2PO_4, 6.7 mM Na_2HPO_4, 5 mM L-glutamic acid, pH 7.4) and stored at -80 °C until use. The numbers of infectious progeny were determined as IFUs by counting chlamydial inclusions formed in HEp-2 cells, using a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody specific to *Chlamydia* LPS (with Evans Blue) (Denka Seiken) [25].

Infection of cells

HeLa and HEp-2 cells, adjusted to a concentration of 2×10^5 cells/well, were infected with bacteria at a MOI of 1 (*C. trachomatis*) or 3 (*C. pneumoniae*), respectively, followed by centrifugation (800×*g*, 30 min) at room temperature. After washing to remove redundant bacteria with Hank's balanced salt solution (Sigma), infected cells at a concentration of 2×10^5 cells/well were seeded into 24-well plates, and then incubated for up to 48 h (*C. trachomatis*) or 72 h (*C. pneumoniae*) in the presence or absence of various drugs (working concentration: 1–100 μg/ml capsaicin, 10 μM capsazepine, 15 μM 5CPPSS-50, 10 μM T0070907, 2 μg/ml cycloheximide). Cells were collected in a time-dependent manner for assessing IFU numbers, chlamydial 16S rDNA quantities, inclusion formation, and bacterial morphological changes. In addition, using a cell counting kit-8 (Dojindo), it was confirmed that these drugs, used at the working concentrations, exerted minimal cytotoxic effects on the cells over the culture period.

Assessment of inclusion formation

Cells were collected to determine the morphology of chlamydial inclusions, as described previously [25]. In brief, after the cells were fixed onto a slide with ethanol, they were stained with FITC-conjugated monoclonal anti-*Chlamydia* antibody specific to *Chlamydia* LPS (with Evans Blue) (Denka Seiken). Cells stained with specific antibodies were observed using a conventional [IX71 (Olympus)] or a confocal laser microscope [LSM510 (Carl Zeiss Japan Group)].

Transmission electron microscopy (TEM)

TEM was performed in accordance with the following method. In brief, cells

were immersed in a fixative containing 3% glutaraldehyde in 0.1 M PBS pH 7.4, for 24 h at 4 °C. After briefly washing with PBS, cells were processed for alcohol dehydration and embedded in Epon 812. Ultra-thin sections of cells were stained with lead citrate and uranium acetate before viewing by TEM (Hitachi H7600; Hitachi). The Hanaichi Ultrastructure Research Institute prepared the ultra-thin sections and carried out observations by TEM.

Quantitative (q) PCR

DNA was extracted from the cultured cells using the LaboPass™ Tissue Mini kit (Hokkaido System Science). PCR was performed using primer sets specific to chlamydial 16S rDNA (which recognize a wide range of chlamydiae) (sense primer, 5′-GGA CCT TAG CTG GAC TTG ACA TGT-3′; antisense primer, 5′-CCA TGC AGC ACC TGT GTA TCT G-3′) [27] and glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) (sense primer: 5′-AAC GGG AAG CTC ACT GGC ATG-3′, antisense primer: 5′-TCC ACC AAC CTG TTG CTG TAG-3′) [28]. The number of bacteria per culture was expressed as the ratio of chlamydial 16S rDNA:*gapdh*.

Assessment of cell death

Cell death was estimated by changes in nuclear morphology and the appearance of condensed chromatin using DAPI staining.

Assessment of mitochondrial membrane integrity

HeLa cells were incubated with capsaicin (0–200 μg/ml) or staurosporine (10 μM) for 48 h, and then incubated with 100 nM MitoTracker Red CMXRos (Invitrogen) for 30

min at 37 °C according to the manufacturer's instructions. After fixing in 4% paraformaldehyde, the cells were observed under a fluorescence microscope.

Western blot analysis

Cells were collected and then boiled for 5 min at 100 °C in a reducing sample buffer containing 2-mercaptoethanol. Then, the samples were separated by 10% (w/v) SDS-PAGE, and the separated proteins were transferred to polyvinylidene difluoride membrane. Membranes were blocked with 5% (w/v) skim milk in Tris-buffered saline and then incubated with an anti-PARP (poly (ADP-ribose) polymerase) antibody (Roche Diagnostics), TRPV1 rabbit polyclonal antibody, α-tubulin monoclonal antibody (Sigma) or rabbit IgG as a control (Sigma) for 1 h at room temperature, followed by a HRP-conjugated goat anti-rabbit IgG (KLP) or goat anti-mouse IgG and IgM antibody (Jackson ImmunoResearch) for 1 h at room temperature. Labeled proteins were visualized with Pierce ECL western blotting substrate, according to the manufacturer's protocol (Thermo Fisher Scientific).

Statistical analysis

Comparisons among groups (more than three) were performed by a multiple comparison test for parametric analysis using the Bonferroni/Dunn method. Comparison between two groups was also performed by the Student's *t* test. A *p*-value of less than 0.05 was considered significant. Calculations were performed in Excel for Mac (2011) with Statcel3C.

Results

Chlamydial growth in HeLa cells in the presence of capsaicin

We first compared the growth of *C. trachomatis* in HeLa cells in the presence or absence of capsaicin. Conventional fluorescent microscopic observations revealed that compared with the control, capsaicin exposure drastically restricted the expansion of chlamydial inclusion size, although there was no difference of the number of infected cells in the presence or absence of capsaicin (Fig. 1A, Green spots). An IFU assay confirmed that the number of infectious progeny (EB) significantly decreased following capsaicin exposure in a dose-dependent manner (Fig. 1B, Black bar); capsaicin exposure similarly inhibited the growth of *C. pneumoniae* in HeLa cells, indicating that this phenomenon is not specific to the *C. trachomatis* strain used in this study (Fig. 1B, White bar). Furthermore, qPCR data revealed that, consistent with the inclusion morphology and IFU data, the amount of chlamydial 16S rDNA significantly decreased following capsaicin exposure in a dose-dependent manner, indicating that no persistent infection occurred (Fig. 1C). These data suggested that capsaicin exposure suppressed *C. trachomatis* infection in HeLa cells as well as *C. pneumoniae* infection.

To confirm this, morphological changes in *C trachomatis* in the presence of capsaicin were assessed by confocal laser microscopy and TEM. Consistent with the results of conventional microscopy, confocal laser microscopic observations showed that chlamydial inclusion size diminished in a dose-dependent manner and no typical inclusion bodies were seen particularly after high-dose exposure of capsaicin (100 μg/ml) (Fig. 2). TEM observations also revealed that, compared with the control (Fig. 3A), the number of bacteria in inclusions formed in the infected cells was limited in the presence of capsaicin and few re-differentiated EB in inclusions were seen (Fig. 3B);

EB (relatively small size and high density) and RB (large size and pale density) were observed with distinct morphological traits. Furthermore, detached bacterial cell walls (Fig. 3C) and disrupted bacteria in inclusion bodies (Fig. 3D) were also observed. Thus, the chlamydial cell structure in the infected cells was morphologically impaired by treatment with capsaicin.

Taken together, these results indicated that capsaicin could inhibit chlamydial growth, through blocking the maturation of the bacteria following bacterial disruption.

Mechanism by which capsaicin inhibits chlamydial growth

It has previously been reported that capsaicin can induce apoptotic cell death via mitochondria dysfunction and caspase-3 activation in immortal cell lines such as MCF-7 or HeLa cells [29, 30]. We therefore assessed whether apoptosis induced by capsaicin exposure was involved in the inhibition of chlamydial growth. When compared with the control using staurosporine as a general apoptosis inducer, the increase in the number of cells with condensed chromatin visualized by DAPI staining was minimal at a working concentration of capsaicin after 48 h incubation, although a slight increase was seen at a higher working concentration (125 μg/ml) (Fig. 4A). Thus, at the usual working concentration of capsaicin, cell death was minimal. To confirm this, we determined the minimal concentration of capsaicin inducing apoptosis of HeLa cells using a more sensitive western blot-based approach to detect PARP cleavage, a critical step in the induction of apoptosis [31]. No PARP cleavage was detected in the presence of less than 100 μg/ml capsaicin, which was the working concentration used in this study (Fig. 4B). In addition, MitoTracker staining revealed that following exposure to capsaicin (100 μg/ml) mitochondrial membrane integrity was maintained, supporting

the findings from western blot analysis above (Online Resource 1). Collectively, these results indicated that the effect of apoptosis induction following capsaicin exposure on the inhibition of chlamydial growth was minimal.

Recent studies have revealed that capsaicin modulates cellular dynamics by actin remodeling via capsaicin receptor TRPV1 [18, 23] and exerts anti-inflammatory effects via PPARγ [18, 24] and LXRα [18, 24]. Specifically, TRPV1 activation altered F-actin organization in an ERK1/2- and MLC2-dependent manner [23], and the inhibitory action of capsaicin on LPS-induced IL-1β, IL-6 and TNF-α production was dependent on the activation of both PPARγ and LXRα [24]. We therefore assessed, using drugs that block the signal transduction of capsaicin [10 μM capsazepine (antagonist for TRPV1), 15 μM 5CPPSS-50 (inhibitor for LXRα) and 10 μM T0070907 (inhibitor for PPARγ)], whether the effects on cellular dynamics by actin remodeling and the anti-inflammatory effects induced by capsaicin exposure were involved in chlamydial growth inhibition. None of the drugs were able to completely restore chlamydial growth following capsaicin exposure, although a slight improvement in growth was detected (Fig. 5). In addition, we confirmed TRPV1 expression on the HeLa cells used in this study by western blotting with TRPV1 rabbit polyclonal antibody (Online Resource 2). Since none of the drugs had an effect on chlamydial inhibition in the presence of capsaicin, capsaicin may inhibit chlamydial growth, presumably with minimal requirement for host responses. To confirm this, we examined whether chlamydial growth inhibition induced by capsaicin exposure could be recovered by stimulation with cycloheximide (2 μg/ml), a eukaryote-specific protein synthesis inhibitor. As expected, regardless of the presence of the drug, capsaicin exposure

similarly inhibited chlamydial growth (Fig. 6).

Taken together, we concluded that capsaicin inhibited chlamydial growth by blocking bacterial maturation following bacterial disruption, but with minimal requirement for host responses.

Discussion

The use of natural compounds that can be easily and safely ingested as part of a daily diet is a new approach to prophylactically overcome a variety of infectious risks. One such compound that has been well investigated is capsaicin (8-methyl-N-vanillyl-6-nonenamide: MW 305.41), a small phenolic compound with hydrophobicity that is produced by all plants including chili peppers [18-22]. Several studies have reported that capsaicin can inhibit the growth of human pathogenic bacteria such as cell-invasive group A streptococci [20], *V. cholera* [21], *E. coli* O157:H7 [22] and *S. enterica* serovar Typhimurium [22]. Here, for the first time, we showed that capsaicin directly inhibited the growth of *C. trachomatis*, an obligate intracellular pathogen, in human immortal epithelial HeLa cells, with minimal requirement for host responses.

An IFU assay revealed that capsaicin dramatically diminished the number of chlamydial infectious progeny, and morphological observations by fluorescence microscopy and TEM revealed that, compared with the control, capsaicin exposure drastically restricted the expansion of chlamydial inclusions. However, there was no difference in the number of infected cells in the presence or absence of capsaicin (see Fig. 1A). Furthermore, pretreatment of the bacteria with capsaicin had no effect on

chlamydial growth inhibition (data not shown). Thus, the results indicated that capsaicin worked against the bacteria in HeLa cells rather than by blocking bacterial attachment to host cells. As mentioned above, it appears that, due to its hydrophobic nature [18], capsaicin can easily penetrate bacteria across first the cellular membrane and then the inclusion membrane. Interestingly, capsaicin exposure also inhibited the growth of *C. pneumoniae* in HeLa cells, indicating that this phenomenon is not specific to the *C. trachomatis* strain used in this study. Meanwhile, the effects of capsaicin on the inhibition of *C. pneumoniae* growth into the IFU assessment were weak with only one log reduction compared with those on *C. trachomatis*; however, the reason for this remains to be clarified. It is possible that because of the slow growth rate of *C. pneumoniae* capsaicin may target the metabolic active site of the bacteria that is required for growth. Furthermore, although further studies should be required for clarifying clinical aspect of capsaicin against chlamydial infection, it implies that capsaicin also has a potential to component to treat *C. pneumoniae*, causing pneumonia with chronic disorder such as asthma [32].

Interestingly, TEM observations revealed that capsaicin not only affected bacterial inclusion size by blocking bacterial maturation, but also caused bacterial morphological changes involving cell wall detachment and bacterial disruption. Our previous studies similarly showed that treatment of HEp-2 cells with a steroid receptor antagonist RU486 (mifepristone), which is a small hydrophobic molecule, significantly inhibited the growth of *C. pneumoniae* in a dose-dependent manner with disruption of the bacteria due to direct binding of the drug to the bacteria [33]. Furthermore, we found that 2-amino-phenoxazine-3-one (phenoxazine derivate, Phx-3: a small hydrophobic molecule) induced the destruction of *C. pneumoniae* in inclusion bodies in immortal

311 epithelial HEp-2 cells, resulting in bacterial growth inhibition [34]. Interestingly, since
312 chlamydial maturation in inclusion bodies is required for manipulation of infected host
313 cells through several effector molecules secreted by type III machinery [35], the
314 dysfunction of type III secretion machinery by the binding of small hydrophobic
315 molecules impaired chlamydia growth in host cells [36]. Although more studies are
316 needed to clarify the mechanisms by which capsaicin causes bacterial morphological
317 changes, it may be that the interaction of small hydrophobic molecules with unknown
318 receptors on the bacterial cell wall is responsible for the dysfunction in bacterial
319 maturation.

320 It is well established that capsaicin evokes several host responses including
321 apoptosis [29, 30], actin remodeling [18, 23] and anti-inflammatory effects [18, 24].
322 However, neither cleaved PARP, mitochondrial dysfunction nor recovery of chlamydial
323 growth inhibition in the presence of inhibitors, capsazepine, 5CPPSS-50 and T0070907,
324 was observed with a working concentration of capsaicin. Furthermore, regardless of the
325 presence or absence of cycloheximide, capsaicin similarly inhibited chlamydial growth.
326 Thus, although at present the mechanism by which bacterial growth is inhibited in the
327 presence of capsaicin remains unknown, we concluded that the influence of host
328 responses stimulated by capsaicin on growth inhibition was minimal. In addition, the
329 presence of the inhibitors along with capsaicin boosted chlamydial growth inhibition,
330 although at present the reason for this remains to be clarified. Because no effect was
331 seen with either inhibitor alone (data not shown), these inhibitors may help capsaicin to
332 access to the bacteria by crossing the membrane.

333 It is well recognized that capsaicin, responsible for the "hot" taste of chili
334 peppers and chili pepper extracts, is a valuable pharmacological agent with therapeutic

applications in controlling pain and inflammation [18-22]. However, although orally administered capsaicin is absorbed into the intestinal and stomach tissues [37], the majority of adsorbed capsaicin is rapidly metabolized in the liver with 16-hydroxycapsaicin, 17-hydroxycapsaicin or 16,17-dehydrocapsaicin, and no intact capsaicin was detected in the tissues or bloodstream four days after oral administration [38]. Drug delivery strategies to improve the bioavailability and therapeutic effects of capsaicin have been investigated and have shown that nanoparticles or liposomes can be used to efficiently deliver intact capsaicin to the bloodstream [39, 40]. Thus, while capsaicin is a promising component to treat *C. trachomatis* infection (presumably also against *C. pneumoniae* infection), further studies are needed to clarify whether dietary ingestion of capsaicin using an appropriate delivery system could target chlamydia infection of the vaginal tissue and/or other sites such as respiratory tract.

In conclusion, we found that capsaicin, a component of chili pepper, can block chlamydial growth (*C. trachomatis* and *C. pneumoniae*) without the requirement of host cell protein synthesis by an, as yet, undetermined mechanism.

Conflict of interest

None.

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Appendix A. Supplementary data

Online Resource 1: MitoTracker staining showing mitochondrial membrane integrity after exposure to capsaicin at 100 μg/ml. Normal mitochondria showed strong red staining compared with abnormal mitochondria (10 μM staurosporine). Magnification, ×600.

Online Resource 2: Western blotting showing TRPV1 expression on HeLa cells.

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Figure legends

Fig. 1. Representative conventional fluorescence microscopic images showing inclusion formation and growth of chlamydiae in HeLa cells in the presence or absence of capsaicin at 48 h after infection. **A**: *C. trachomatis*-infected cells in the presence or absence of capsaicin (0–100 μg/ml) were stained with FITC-conjugated anti-*Chlamydia* antibody. The images were captured at 48 h after infection. Green spots, inclusion bodies. Control, diluted DMSO. Magnification, ×400. **B**: Number of chlamydial infectious progeny were estimated at 48 h (*C. trachomatis*) or 72 h (*C. pneumoniae*) after infection by an IFU assay. Control, diluted DMSO. Ct, *C. trachomatis*. Cp, *C. pneumoniae*. Data show the mean + SD. *p<0.05 versus the value of each of the controls. **C**: Amount of *C. trachomatis* 16S rDNA was estimated by qPCR. Data are expressed as the ratio of 16S rDNA:*gapdh*. See the Materials and methods. Data show the mean + SD. *p<0.05 versus the value of the control.

Fig. 2. Representative confocal laser fluorescence microscopic images showing inclusion formation and growth for *C. trachomatis* in HeLa cells in the presence or absence of capsaicin at 48 h after infection. *C. trachomatis*-infected cells in the presence or absence of capsaicin (0–100 μg/ml) were stained with FITC-conjugated anti-*Chlamydia* antibody. Blue, DAPI. Green spots, inclusion bodies. Control, diluted DMSO.

Fig. 3. Representative TEM image showing the bacterial morphological traits in

inclusion bodies of *C. trachomatis* formed in HeLa cells at 48 h after infection. Arrows show inclusion bodies. **A:** Control, diluted DMSO. **B–D:** + capsaicin, infected cells were incubated with 100 μg/ml capsaicin. Arrows show representative EBs. Stars show representative RBs.

Fig. 4. Influence of apoptosis induced by capsaicin exposure on the growth inhibition of *C. trachomatis*. Cells were analyzed at 48 h after infection according to the following traits. **A:** DAPI-stained cells were analyzed for morphological changes including condensed chromatin. Staurosporine, which induces apoptosis, was included as a positive control. Data show the mean + SD (%). **p*<0.05 versus the value for the staurosporine control. **B:** Representative western blot images showing the induction of apoptosis by capsaicin exposure. Open arrow shows PARP cleaved by apoptosis induction. Staurosporine treatment was included as a positive control.

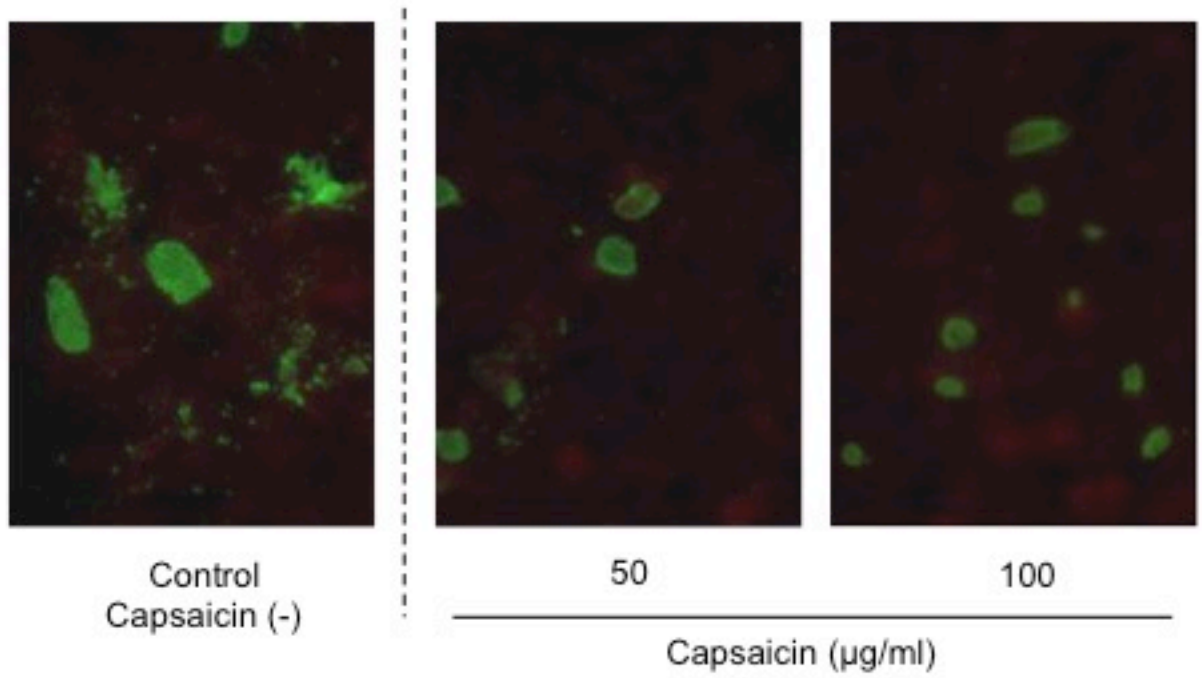
Fig. 5. Influence of inhibitors against capsaicin on chlamydial growth inhibition following capsaicin exposure. HeLa cells with or without *C. trachomatis* infection were incubated for up to 48 h in the presence or absence of inhibitors [10 μM capsazepine (antagonist for TRPV1), 15 μM 5CPPSS-50 (inhibitor for LXRα), 10 μM T0070907 (inhibitor for PPARγ)] with or without capsaicin (50 μg/ml). The number of infectious progeny was estimated by an IFU assay at 48 h after infection. Control, diluted DMSO. Data show the mean + SD. **p*<0.05 versus the value of the control.

Fig. 6. Influence of cycloheximide (eukaryote-specific protein synthesis inhibitor) on

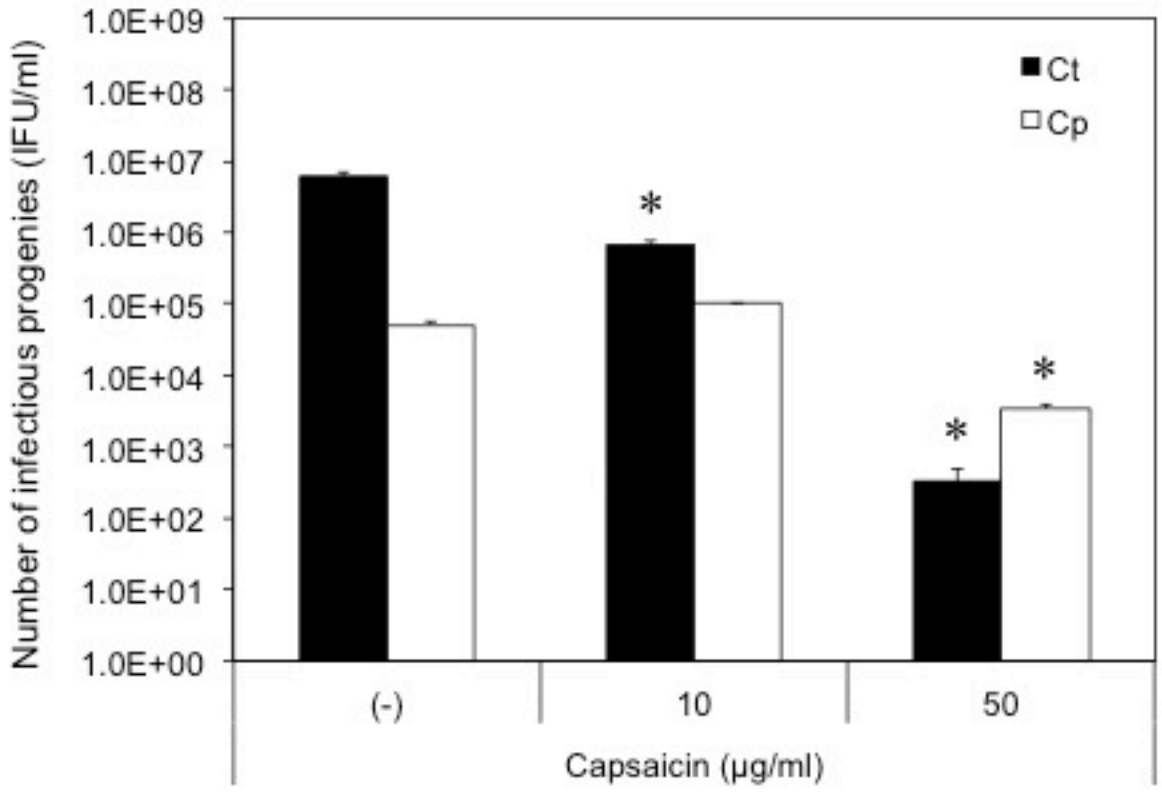
chlamydia growth inhibition following capsaicin exposure. *C. trachomatis*-infected cells were incubated in the presence or absence of cycloheximide (2 μg/ml) with or without capsaicin (50 μg/ml). After incubation, the number of infectious progeny was estimated by an IFU assay at 48 h after infection. Data show the mean + SD. *$p<0.05$ versus the value of each of the controls “Capsaicin (-)”.

Fig. 1

A


Control
Capsaicin (-)
50
100
Capsaicin (μg/ml)

B


Number of infectious progenies (IFU/ml)
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
Ct
Cp
*
*
*
*
(-)
10
50
Capsaicin (μg/ml)

C

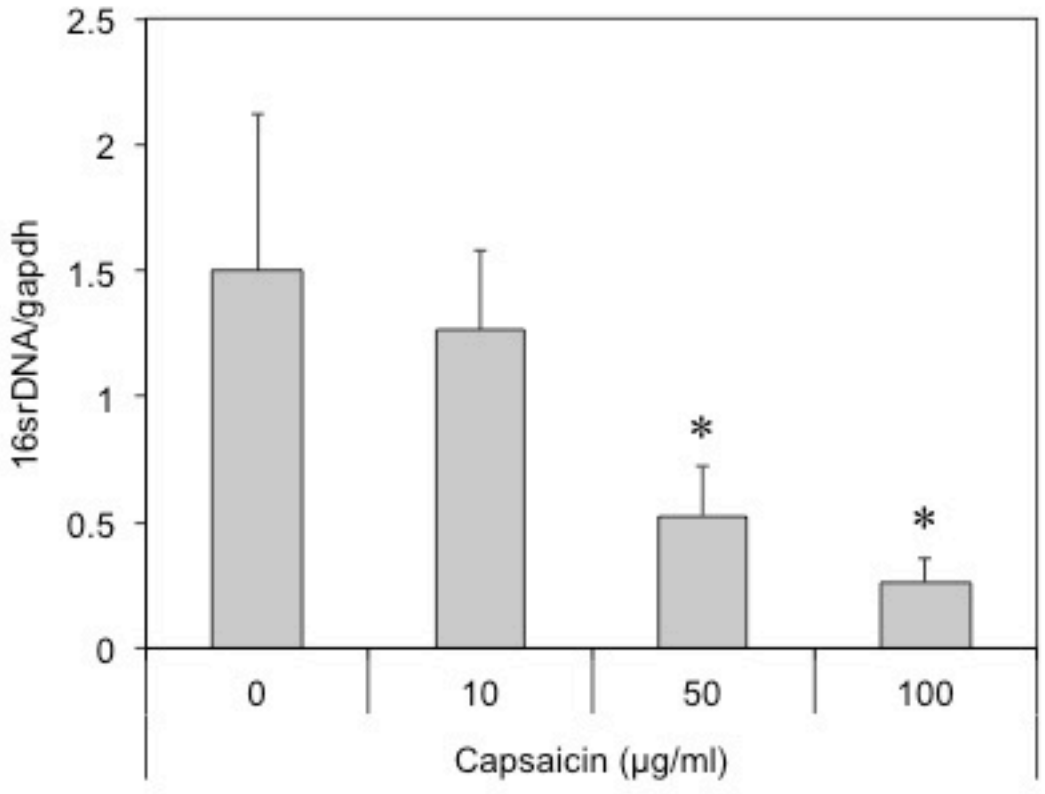

16srDNA/gapdh
2.5
2
1.5
1
0.5
0
*
*
0
10
50
100
Capsaicin (μg/ml)

Fig. 2

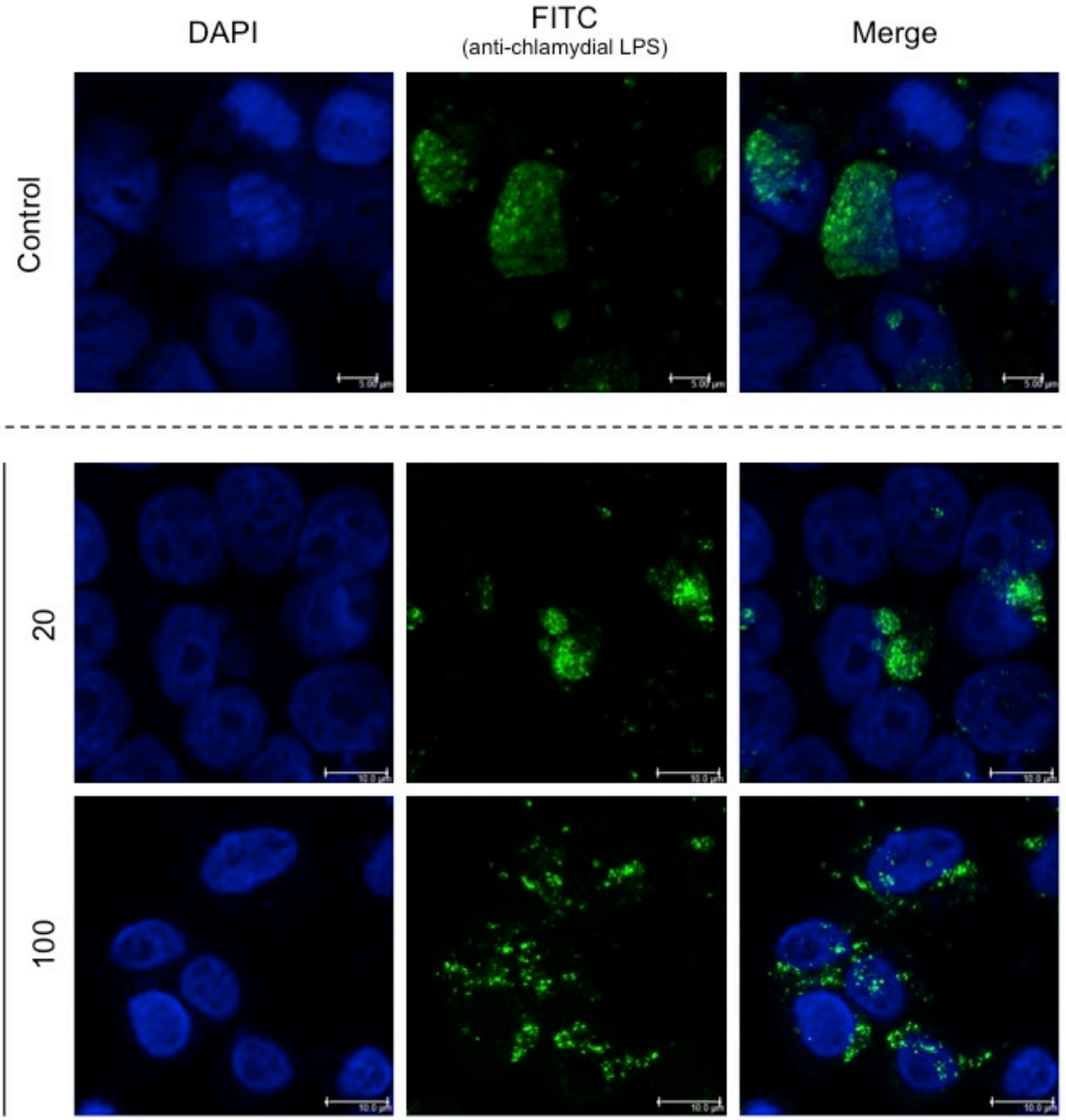

DAPI
FITC
(anti-chlamydial LPS)
Merge
Control
Capsaicin (μg/ml)
20
100

Fig. 3

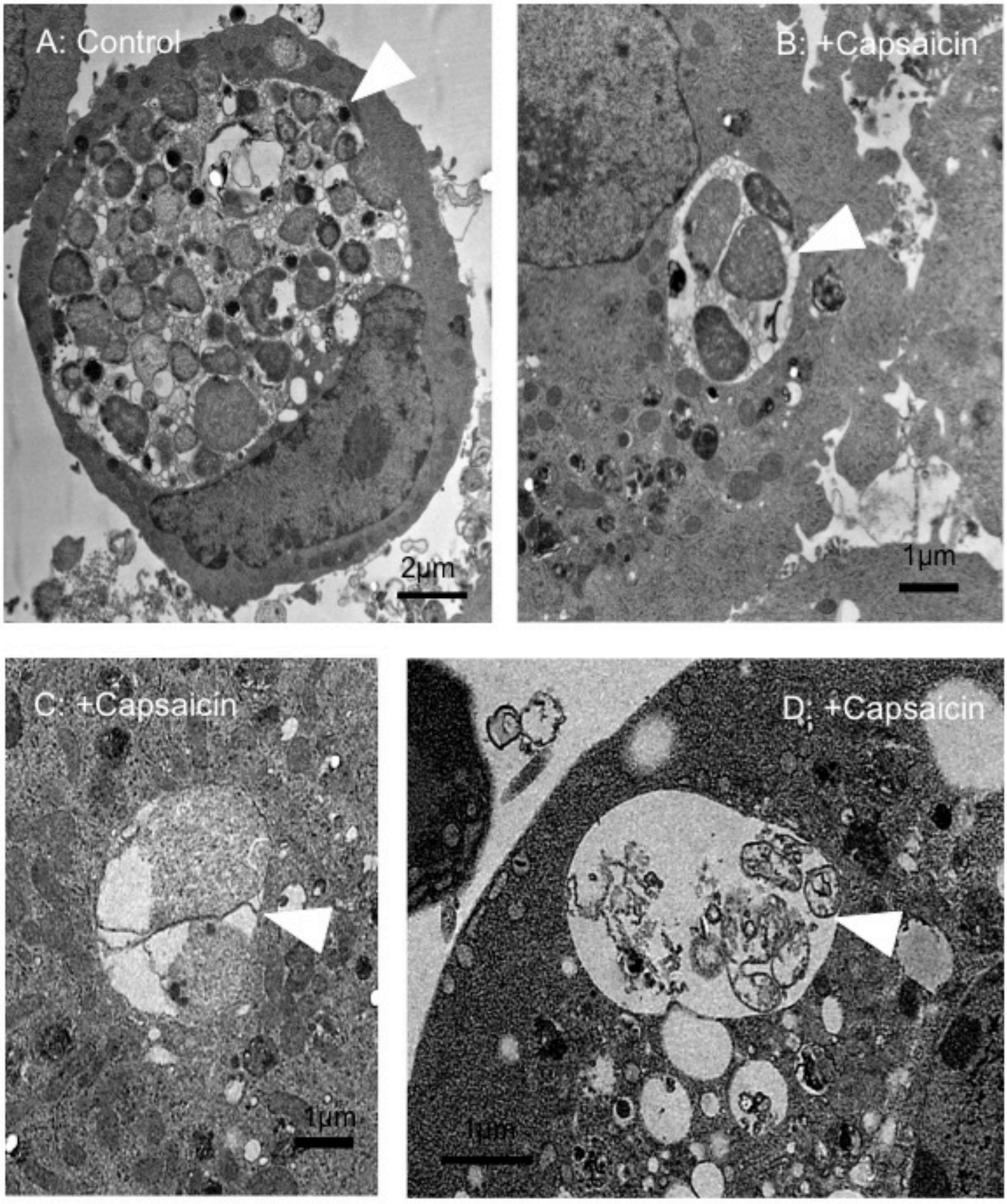
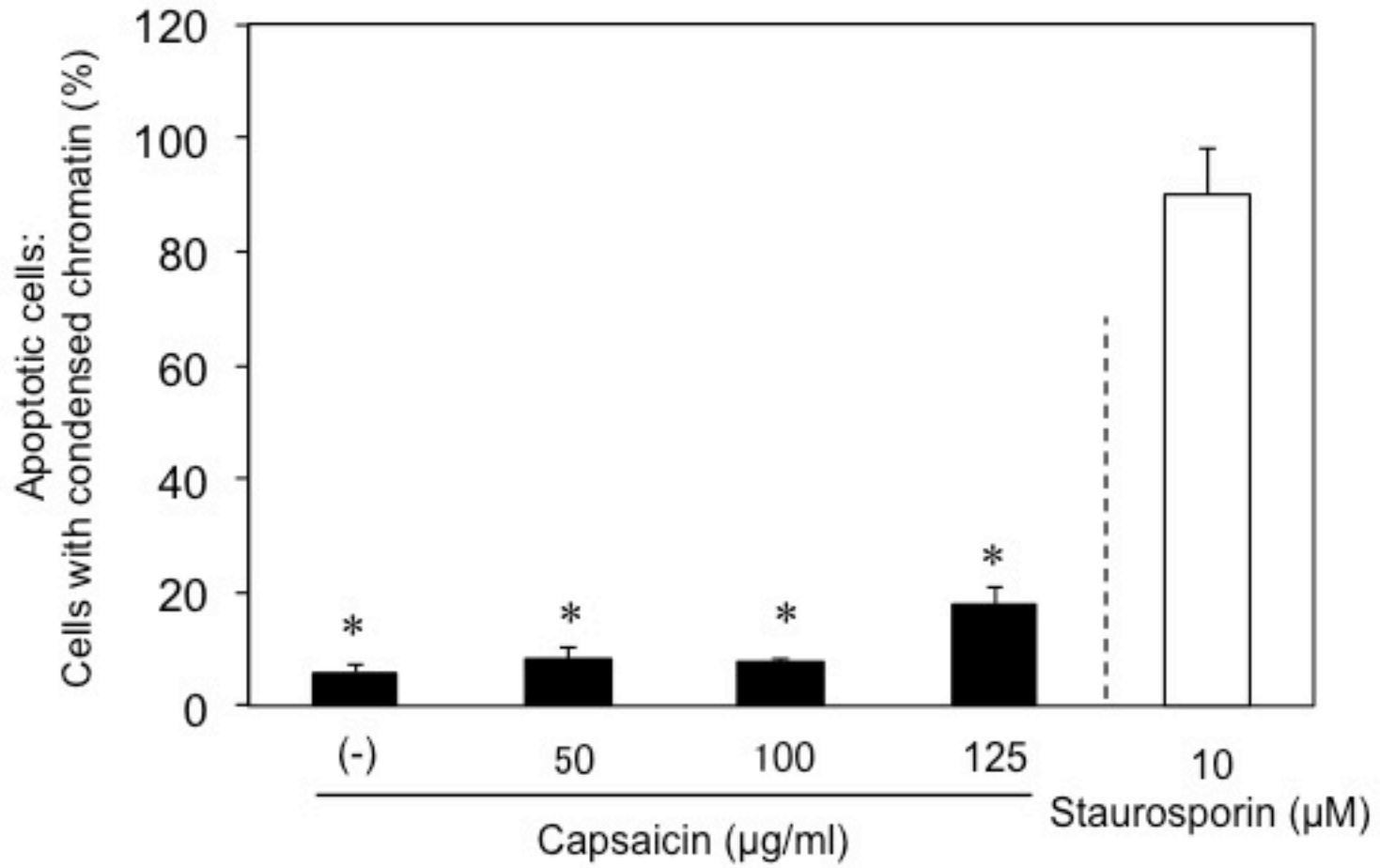

A: Control
2μm
B: +Capsaicin
1μm
C: +Capsaicin
1μm
D: +Capsaicin
1μm

Fig. 4

A


Apoptotic cells:
Cells with condensed chromatin (%)
120
100
80
60
40
20
0
*
*
*
*
(-)
50
100
125
10
Staurosporin (μM)
Capsaicin (μg/ml)

B

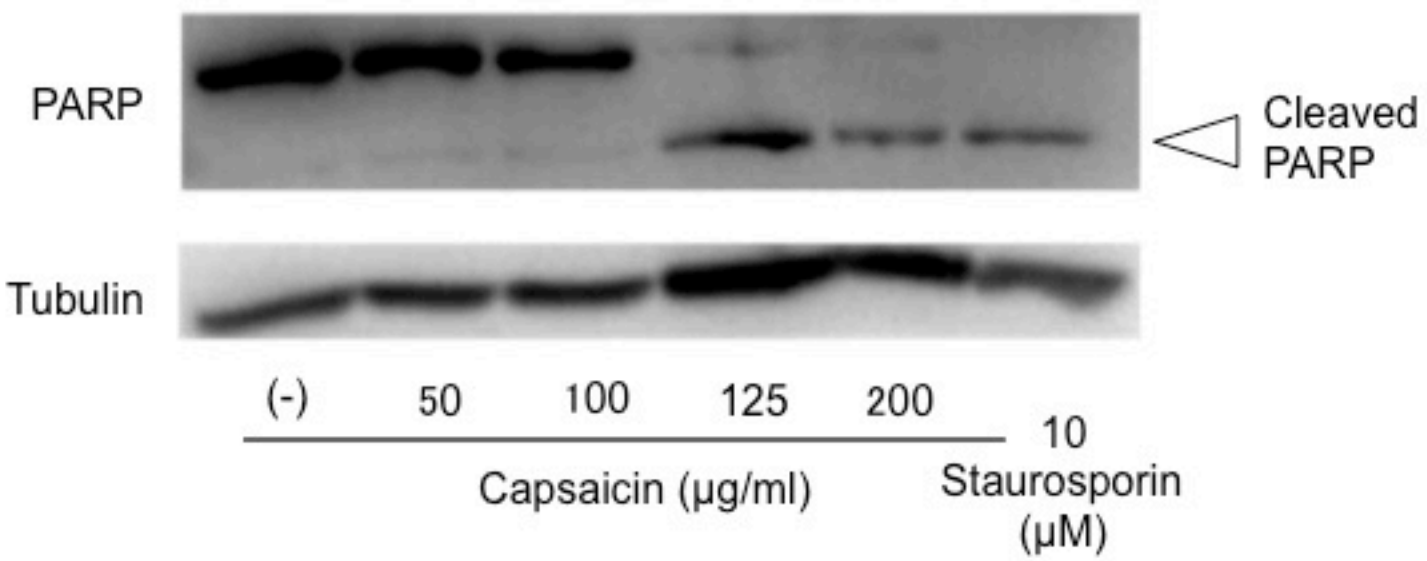
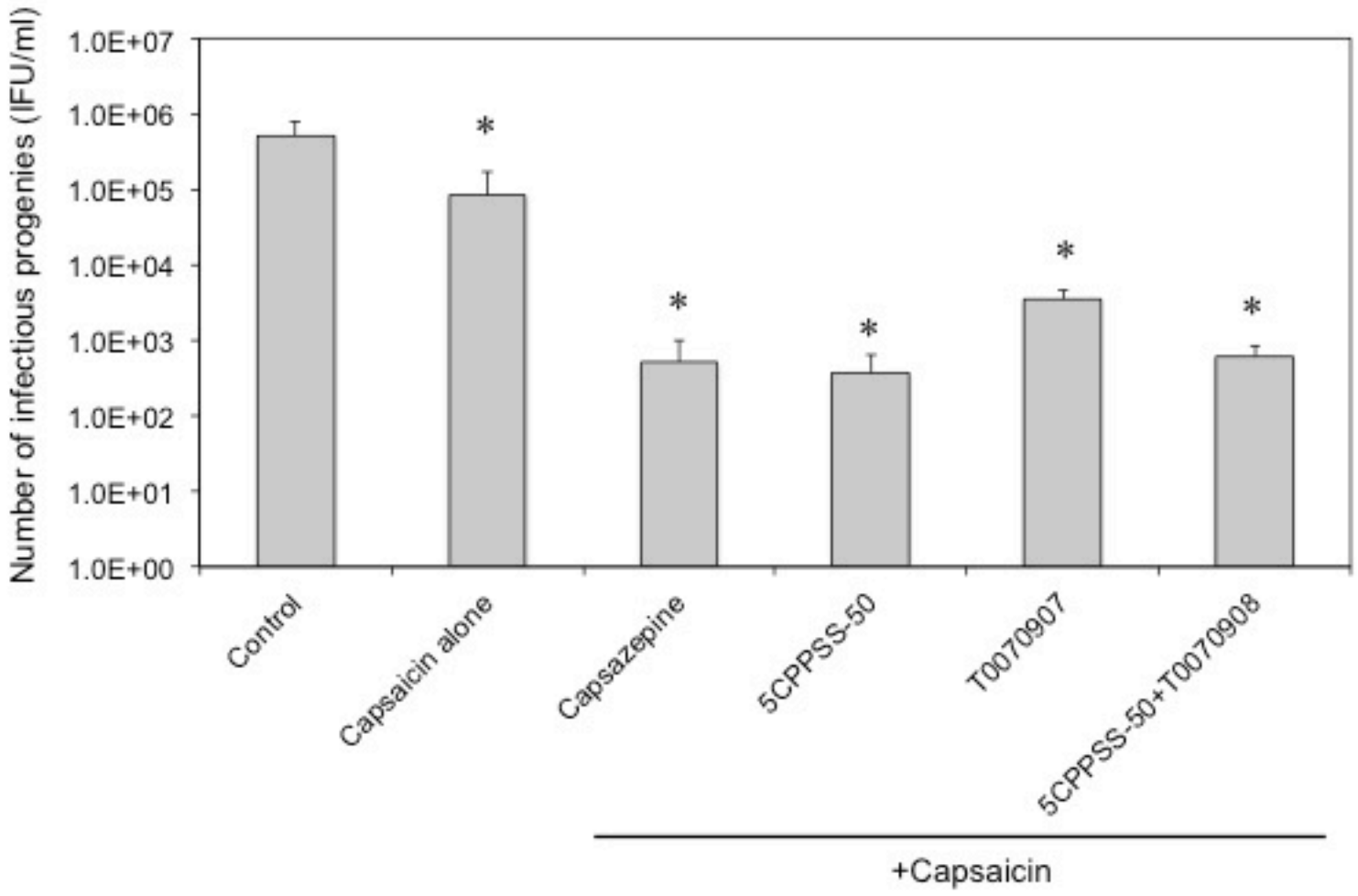
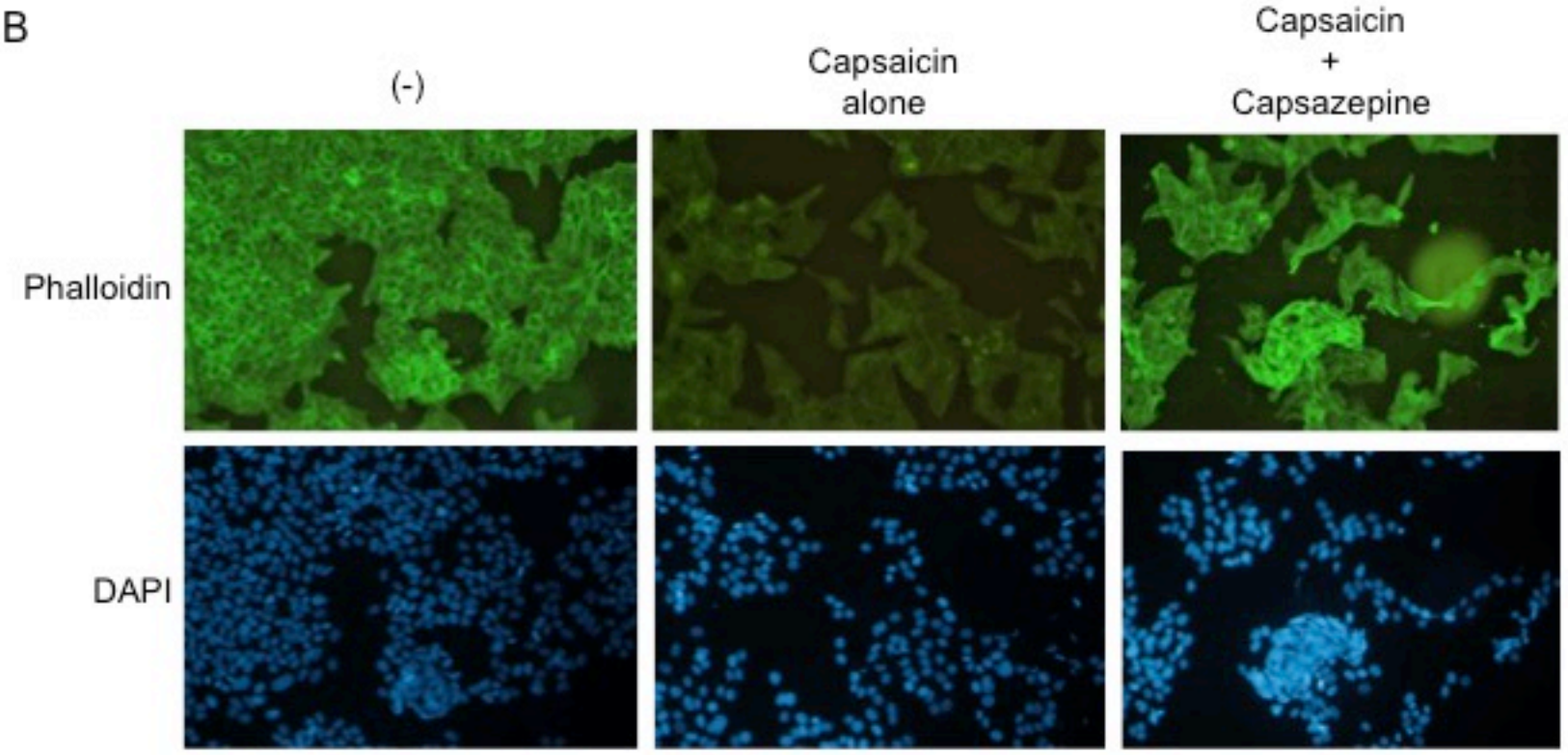

PARP
Cleaved
PARP
Tubulin
(-)
50
100
125
200
10
Staurosporin
(μM)
Capsaicin (μg/ml)

Fig. 5

A


Number of infectious progenies (IFU/ml)
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
*
Control
Capsaicin alone
Capsazepine
5CPPSS-50
T0070907
5CPPSS-50+T0070908
+Capsaicin

B


(-)
Capsaicin alone
Capsaicin + Capsazepine
Phalloidin
DAPI

C

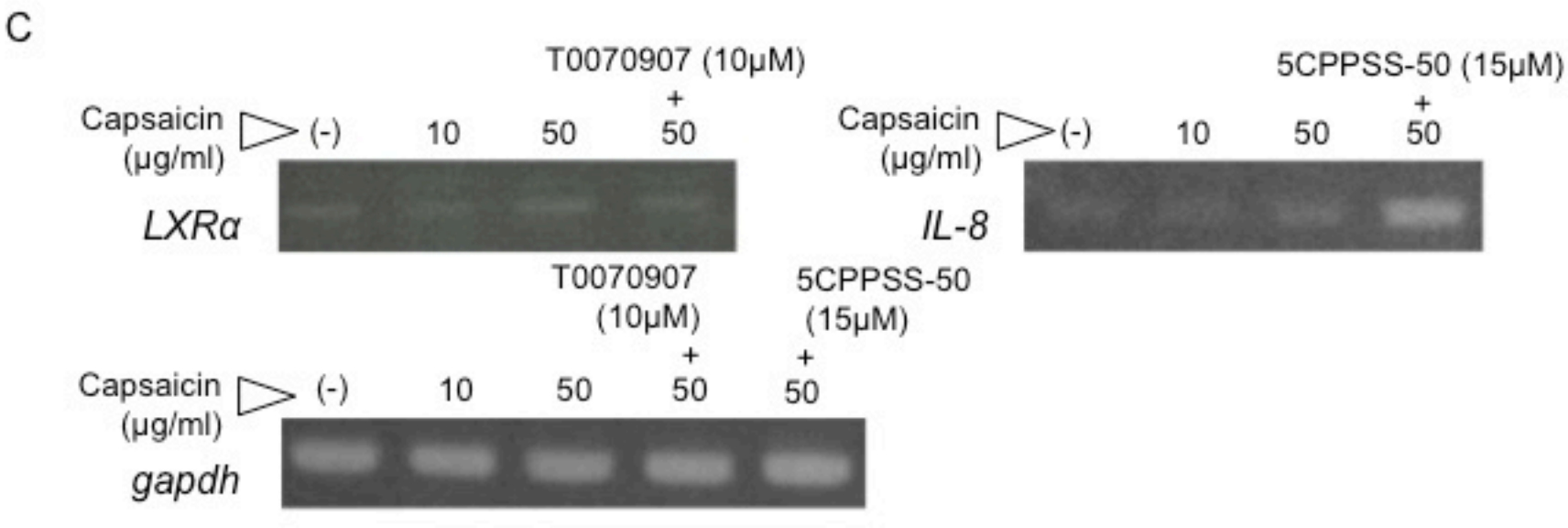
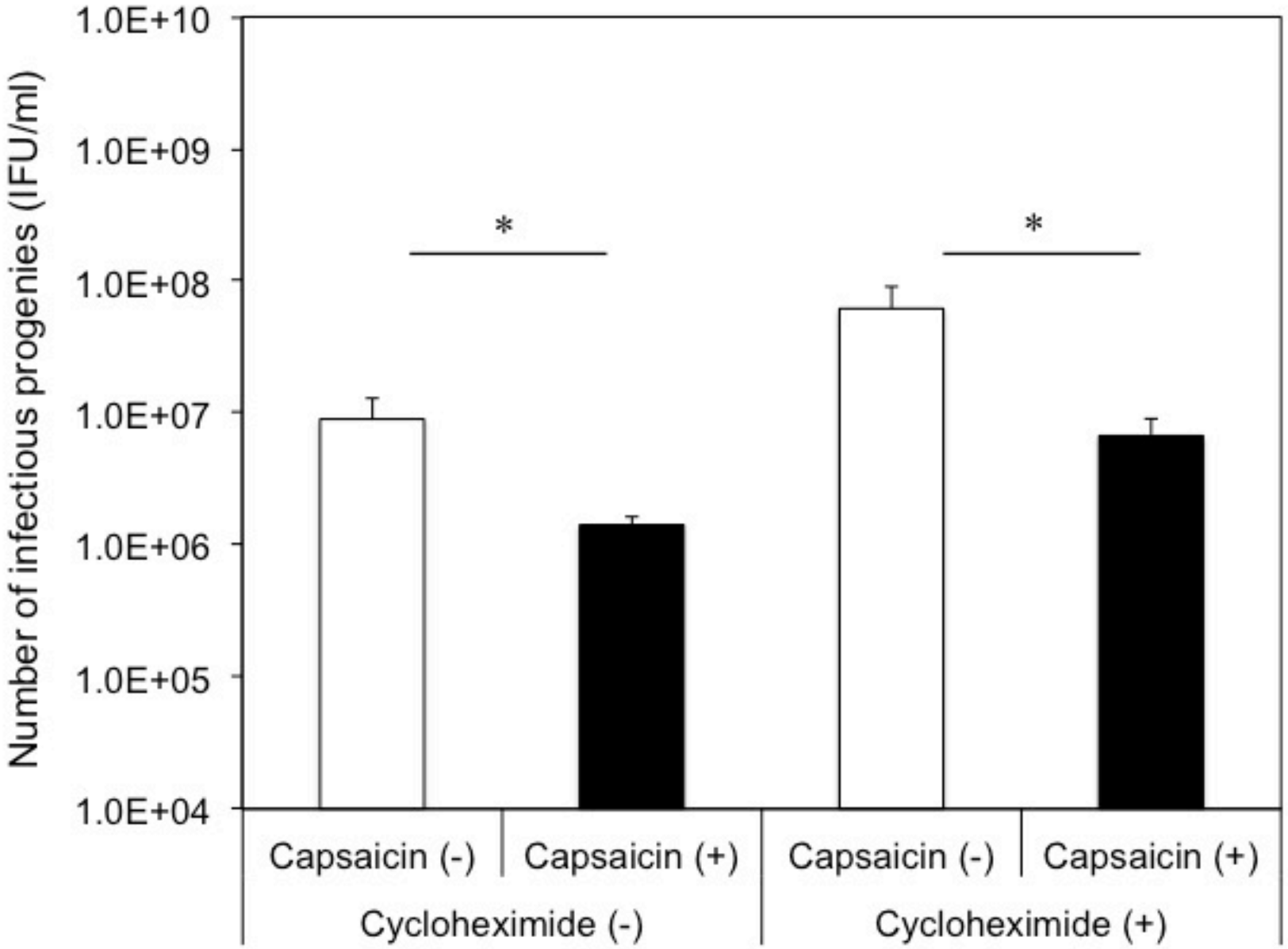

T0070907 (10μM)
+
Capsaicin (μg/ml)
(-)
10
50
50
LXRα
5CPPSS-50 (15μM)
+
Capsaicin (μg/ml)
(-)
10
50
50
IL-8
T0070907 (10μM)
5CPPSS-50 (15μM)
+
+
Capsaicin (μg/ml)
(-)
10
50
50
50
gapdh

Fig. 6


1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
Number of infectious progenies (IFU/ml)
*
*
Capsaicin (-)
Capsaicin (+)
Capsaicin (-)
Capsaicin (+)
Cycloheximide (-)
Cycloheximide (+)