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ARTICLE

# Comparing the effects of staurosporine and ultraviolet radiation on apoptosis in cell culture and *in vivo*.

R. L. O.

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**Apoptosis is a form of programmed cell death that organisms utilize for the regulation of cell function and the systematic maintenance of cellular homeostasis. Both with embryonic development and with adult tissue management, apoptosis has been observed to be a normal part of an organism's life and its self-regulation therein. The purpose of this study was to observe the different effects that staurosporine and ultraviolet radiation had on apoptosis, both with HeLa cell culture and *in vivo* with *Caenorhabditis elegans*. Cell death by apoptosis can be visibly identified (via microscopy) by key morphological characteristics of which were used to compare the effects of the different treatments. A better understanding of apoptosis could one day lead to better characterization of embryonic development and organismal homeostasis that could inform therapeutic medical treatment methods.**

## Introduction

Apoptosis is a required systematic mechanism that organisms utilize for the maintenance of homeostasis and regulation of cell function. Apoptosis is a form of programmed cell death and is highly utilized with normal embryonic development and adult tissue self-regulation. With regard to embryonic development, organisms utilize apoptosis to bring definition to tissues such as limbs and digits. With regard to adults, apoptosis is a common method to destroy and recycle cells that the organism no longer needs. A common example of this is with adult tissues: the organism produces and destroys cells at a steady and relatively equal rate. Another key role that apoptosis plays is as a protective function for when cells have become damaged or infected. The organism utilizes apoptosis to rid itself of these harmful cells (Alberts *et al.* 2015).

HeLa cells are human cells grown in culture and allow for examination of the Intrinsic and extrinsic pathways of apoptosis. Both of these pathways are inducible and utilize the executioner caspase 3 (cysteine-aspartic protease) that performs a major role in the death of the cell. The intrinsic pathway can be triggered by both staurosporine and ultraviolet radiation treatments and begins by Bax and Bak BH123 proteins releasing cytochrome C from the mitochondria. The free cytochrome c activate APAF1 (CED-4 in *Caenorhabditis elegans*) and form apoptosomes. These apoptosomes recruit procaspase 9 molecules of which get cleaved to form caspase 9 molecules. Caspase 9 serves as a caspase cascade initiator that activates caspase 3 and thus executes apoptosis. With regard to the extrinsic pathway, ultraviolet light can stimulate the activation of the extrinsic pathway by stimulating Fas-associated proteins with death domains (FADD) that target the cells that need to die and stimulate the cleaving and activation of procaspase 8 or 10 (or both) to the initiator caspase 8 or 10 or both. The activated initiator caspase 8 or 10 (or both) then proceed down the caspase cascade by activating caspase 3 and thus execute apoptosis. These two pathways can be observed and differentiated in HeLa cells when comparing the two treatments.

*Caenorhabditis elegans (C. elegans)* is a model organism that primarily utilizes only one pathway somewhat similar to the intrinsic pathway. The metazoan *C. elegans* is a model organism for biological study because the organism is well-suited for microscopy because of its relatively transparent body, undergoes consistent cell development, has a fixed number of cells, and has its entire genome mapped with the ability to isolate and reduce the expression of genes by use of RNAi (Corsi *et al.* 2015). *Ced-4* is the homolog for the *Homo sapiens* gene *apaf-1*. CED-4 proceeds to then activate a caspase executioner (CED-3) (Seshagiri and Miller 1997). This pathway was examined and compared between the two treatments, as well as comparing models with an RNAi *ced-4* treatment to observe whether or not apoptosis proceeds or not. Additionally, it is believed that *C. elegans* has an alternative parallel pro-apoptotic pathway to active the CED-3 caspase using DPL-1 and EFL-1/-2 of the retinoblastoma tumor suppressor complex, and that these proteins could contribute to stimulating apoptosis regardless of CED-4 activity (Schertel and Conradt 2007).

With the *in vivo* model, apoptosis activity of *Caenorhabditis elegans* was examined in realtionship to *ced-4* activity. CED-4 (APAF1) is the apoptosome which activates CED-3 (caspase), so silencing it should not see CED-3 activation (Yuan and Horvitz 1992). CED-3 is involved in the execution of apoptosis, so blocking *ced-4* should ultimately result in a phenotype that has zero programmed cell death activity. Most programmed cell death occurs during embryo development, and continues less during adult life. An organism without programmed cell death normally has the following negative effects on the organism: The evidence of undead cells which are those cells that were destined to die by *egl-1* but did not and rather differentiated, functioned, and showed an overall increase of 12% more somatic cell activity. They are also known to have a larger nervous system, grow more slowly, have reduced fertility, and have defects in tasks such as chemotaxis and others. They are otherwise indistinguishable from the wild type and are viable and fertile. *Ced-4* has two isoform transcripts from alternative splicing: CED-$4_S$ which promotes programmed cell death, and CED-$4_L$ which antagonizes programmed cell death (Shaham and Horvitz 1996). It has been observed that *ced-4* RNAi knockdown is embryonically lethal (Cecconi 1999).

Cell death by apoptosis can be visibly identified (via microscopy) by some key morphological characteristics such as blebbing of the plasma membrane, damage and disassembly of actin filaments, disassembly and collapse and fragmentation of the nuclear envelope, and the visible presence of corpse bodies. These features were used when comparing treatments with the control and experimental models. In HeLa cells, apoptotic characteristics were compared between treatments using different fluorescent stains and were used to view actin and nuclear activity, as well as electrophoresis and immunoblotting to observe caspase activity. The transgenic *C. elegans* strain MD701 was used which contains a CED-1::GFP fused protein that allows for the CED-1 sheath cell transmembrane protein to be viewed under fluorescent microscopy. These sheath cells in the gonad region of *C. elegans* allow the formation of apoptotic corpse bodies in these cells to be viewed.

Ribonucleic acid interference (RNAi) was used to isolate and reduce (silence) the expression of *ced-4* in *C. elegans*. The RNAi technique consists of feeding *C. elegans* HT115 *E. coli* that contain plasmids that contain the *ced-4* target gene (pL4440:CED-4 plasmid). The positive control model had *ubc-2* in its plasmid which has a phenotype of embryonic lethality. The vehicle negative control had an empty vector. These plasmid-containing bacteria were fed to the organism and then observed for phenotype comparisons. The mechanism for RNAi involved the transcribing of the plasmids into double-stranded RNA molecules within the organism itself and uses the transcription machinery of the host. The double-stranded RNA gets targeted and cleaved by the DICER protein and then complexed with the RISC complex. This complex then targets and cleaves the host's matching mRNA for that same gene and thus inhibits its translation. Inhibited translation results in silenced gene expression for the specific gene of interest.

The purpose of this study was to observe the different effects that staurosporine (STS) and ultraviolet radiation (UVC) had on apoptosis, both with HeLa cell culture and *in vivo* with *Caenorhabditis elegans*. It was hypothesized that STS will induce apoptosis via the intrinsic pathway in HeLa cells, UVC will induce apoptosis via the extrinsic pathway in HeLa cells, and that CED-4 silencing will prevent apoptosis in *C. elegans* unless the alternative apoptotic pathway (DPL-1 and EFL-1/-2 → CED-3) is utilized. A second hypothesis was that UVC is a better stimulator of apoptosis than STS because it uses both apoptotic pathways (the extrinsic and intrinsic pathways). The main techniques utilized in this study for HeLa cultured cells were the MTT cell viability assay, Phalloidin and DAPI staining, and Western Blot. For the *in vivo C. elegans* organism, the main technique that was used was fluorescent microscopy and RNAi. In this study, it was concluded that STS did induce the intrinsic pathway in HeLa cells (as expected), that UVC did induce the extrinsic pathway (as expected), and that apoptosis was inhibited in *C. elegans* with STS treatment (as expected), but not fully for UVC treatment (not expected).

## Materials and Methods

**HeLa cell culture and cell viability Trypan Blue exclusion assay**

HeLa Cells were acquired from the Middle Tennessee State University Biology Department, were cultured at 37ºC with 5% $CO_2$, and were passaged every 2-3 days. The growth media used was Dulbecco's Modified Eagle's Medium and contained 4.5 g/L glucose, sodium pyruvate, and L-glutamine. The media included 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin and the cells were cultured in a Petri dish cell culture.

Once the cells were grown, adhered HeLa cells were removed from media by washing in phosphate-buffered solution (PBS), adding 200µL of trypsin, and incubating for 5 minutes. Addition of 1800µLs of new media was added to suspend cells in. The cell viability stain Trypan Blue Exclusion Assay was used to measure cell viability. A hemocytometer was used to count the viable cells (colorless) against dead cells (dead cells have a compromised plasma membrane and can take up the Trypan Blue stain) (Green and Sambrook 2012). Percent

Cell Viability was measured at 1.75 x $10^4$ cells/mL with a dilution factor of 1.0 (0.5mL Trypan Blue:0.5mL PBS).

**MTT cell viability assay comparing treatment parameters**

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to measure mitochondrial activity of treated HeLa cells. The assay compared the two treatment parameters: 1. Staurosporine (STS) Treatment; and 2. Ultraviolet C (UVC) radiation. For the STS treatment, the cells had their media removed, washed in PBS, and treated with 2mL of 100nM STS for 18 hours. For the UVC treatment, cells had their media removed, washed in PBS, had 1mL of media added, treated with 10 minutes of UVC light, and had 1mL of media added, and incubated for 18 hours. Both treatments were incubated at 37ºC with 5% $CO_2$ for the 18 hours. A spectrophotometer was used to measure optical density absorbance at 570nm, which represents the amount of formazan present and indicates the relative amount of mitochondrial reductase activity, and was compared between the parameters (Green and Sambrook 2012).

**Phalloidin and DAPI Stain**

Phalloidin stain, which is a fluorophore marker that binds to Actin, and DAPI stain which binds to AT base pairs of DNA, were used to compare apoptotic morphology between staurosporine and ultraviolet C radiation treated HeLa cells. HeLa cells were washed with PBS (pH 7.4), fixed with 4% formaldehyde solution in PBS for 10 minutes, and then washed twice with PBS. Cells were extracted on magtech dishes for 3-5 minutes with 500µL 0.1% Triton X-100 in PBS and then washed twice with PBS.

For staining, prepare each slide in a dilution of 5µL methanolic stock into 200µL PBS. 1% bovine serum albumin was added to the staining solution to reduce non-specific binding (non-specific staining). Cells were incubated for 30 minutes. Phalloidin stain was added to coverslips and covered and incubated for 20 minutes at room temperature. Coverslips were then washed with PBS twice (ThermoFisher Scientific 2016). Stained cells were then viewed on Motic brand 100W mercury illuminator, fluorescent compound microscope with phase contrast.

**RIPA Lysis**

HeLa cells were treated three different parameters: 1. UVC for 15 minutes; 2. STS for 19 hours; and 3. Control (untreated). Adherent HeLa cells were washed twice with ice cold PBS in order to separate cells from media. 1mL of ice cold RIPA Lysis and Extraction Buffer (with protease inhibitor already added) was added to every 5x$10^6$ cells in 75$cm^2$ flasks. Mixtures were then incubated with ice for 10 minutes and intermittently mixed by pipetting. Cell mixture was then centrifuged at approximately 14,000rpms for 15 minutes (Pierce Biotechnology). Supernatant was standardized via BCA and then transferred for use with SDS-Page (Thermo Scientific).

**BCA Standardization**

Supernatant protein solution was standardized using the Pierce BCA (bicinchoninic acid) Protein Assay Kit. The dilution used involved a 9-vial scheme with diluent and bovine serum albumin (BSA) standard as follows: Vial A with 300µL of stock BSA solution; Vial B with 375µL of stock BSA solution and 125µL of diluent; Vial C with 325µL of stock BSA solution and 325µL of diluent; Vial D with 175µL of Vial B dilution and 125µL of diluent; Vial E with 325µL of Vial C dilution and 325µL of diluent; Vial F with 325µL of Vial E dilution and 325µL of diluent; Vial G with 325µL of Vial F dilution and 325µL of diluent; Vial H with 100µL of Vial G dilution and 400µL of diluent; and Vial I with 400µL of diluent. The working reagent (WR) was calculated to be 17mL of Reagent A and 340µL of Reagent B (1:50 dilution of A:B). 25µL of each vial, along with 200µL of the working reagent were added to the microplate for spectrophotometry after 30 minute incubation at 37ºC. Absorbance was measured at 562nm and results were subtracted from the blank standard replicate. A standard curve was extrapolated from a best-fit line from the plotted average results (Thermo Scientific).

**SDS-Page**

The volume of water ($V_w$) needed for dilution was solved to be 0µL by the $C_1V_1 = C_2V_2$ equation. $C_2$ was the lowest concentration sample from the BCA standardization and equaled 107.11ng/mL. $V_1$ (volume of protein sample) was calculated to be 48µL. The amount of Laemmli buffer used to prepare the samples was calculated to be 64µL per 48µL of sample. The sample was then boiled at 90ºC to prepare the proteins and to denature any proteases. The gels were then loaded with the buffer solution and then each lane was loaded with 20-25µL of its respective sample and also with the protein ladder. The gel was run with 100V. Once the gel was completed, the transfer was made to a equillibriate nitrocellulose membrane in 1x transfer buffer (40mM TAE buffer + glycine + 1mM SDS) for 2-3 minutes. The transfer stack was constructed (gel on membrane on pad) and run in the RTA transfer kit for 7 minutes.

**Western Blot**

The transferred membrane from the SDS-Page electrophoresis was stained with a primary antibody stain. Cells on the membrane were washed with 1x TBST and then soaked in 5% milk in 1x TBST for 60 minutes to reduce any non-specific binding. The stains used were rabbit monoclonal anti-human antibodies for caspase 8 (57 kDa Full) and cleaved caspase 8 (43/41/18 kDa), PARP (116 kDa), cleaved PARP (89 kDa), caspase 3 (35 kDa

Full), cleaved caspase 3 (17/19 kDa), and actin (37 kDa) (control) and were added at a 1:4,000 dilution and were incubated at 4ºC overnight in 5% milk in 1x TBST. The secondary antibody stain was then added at a 1:3,000 dilution. The stain used was a mouse anti-rabbit antibody that contained the horseradish peroxidase (HRP) enzyme. Electrogenerated Chemiluminescence (ECL) 1 and 2 were then added and then imaged with Chemidoc.

***C. elegans* growth and maintenance**

The MD701 transgenic strain was grown in Nematode Growth Medium agar at 25ºC and fed OP50 (uracil auxotroph *E. coli* strain). Experimental parameters were as follows for the first *C. elegans*: the worms used in the first experiment (not using RNAi) were fed OP50 and treated with 1 of 4 parameters: 1. UVC for 15 minutes; 2. .5µM STS in .1µM DMSO (1:1000 dilution in 100µL) for 12 hours and then were imaged 12 hours after treatments; 3. .1µM DMSO only (negative control); 4. Untreated (second negative control). Worms were imaged on a ZEISS AxioObserver confocal microscope with a a laser scanning microscopy 700 module and a DIC M27 objective (63X/1.4 Plan-Apochromat oil).

**RNAi Knockdowns**

L4-stage MD701 worms used in the second *C. elegans* experiment (RNAi knockdown experiment) and were treated with 1 of 6 parameters: 1. Fed HT115 *E. coli* with empty plasmid and treated with UVC; 2. Fed HT115 *E. coli* with empty plasmid and treated with STS; 3. Fed HT115 *E. coli* with empty plasmid and treated with .1µM DMSO only (negative control); 4. Fed HT115 *E. coli* with pL4440:CED-4 plasmid (RNAi) and treated with UVC for 15 minutes; 5. Fed HT115 *E. coli* with pL4440:CED-4 plasmid (RNAi) and treated with .5µM STS in .1µM DMSO (1:1000 dilution in 100µL) for 12 hours; 6. Fed HT115 *E. coli* with pL4440:CED-4 plasmid (RNAi) with no treatment (negative control). All parameters were imaged with confocal 12 hours after treatments.

## Results

With regard to the MTT cell viability assay, cells that were treated with 10 minutes of UVC light show a substantial decrease in cell viability as compared to those treated with STS and the untreated control (Figure 1). Cells treated with STS show a decrease in cell viability when compared to the untreated control. The UVC treatment caused a statistically relevant decrease in cell viability when compared to staurosporine (Figure 1). Two-tailed *t* tests confirm that these differences between UVC and the untreated control are statistically significant ($P < 0.0001$) and between STS and the untreated control ($P = 0.0012$) (GraphPad Software).

For the Phalloidin and DAPI stains, the control model showed normal HeLa cells intact with normal morphology (Figure 2). The HeLa cells treated with UVC and STS both show fragmentation and amorphic shaping of the nuclear envelope. They also show blebbing and fragmentation of the actin filaments. The UVC treatment appeared to cause a greater apoptotic effect on the nucleus while STS caused a more visible effect on the actin filaments.

With regard to the lysed cells from the RIPA lysis procedure that were used for the SDS-Page electrophoresis, the BCA standardization curve was calculated from a best fit trendline from the plotted data and rendered a standardized protein sample concentration of 107.11ng/mL (Figure 3). With regard to the resulting Western Blot from the protein samples, Cleaved caspase-8 was only in the UVC treated cells, and was not observed in the control or STS treated HeLa cells (Figure 4a.). PARP was observed to be cleaved in UVC and STS treatments (Figure 4b.). In UVC and STS treatments, caspase-3 was observed to be cleaved (Figure 4c.). The results were verified by compared with the control (actin) (Figure 4d.).

*C. elegans* MD701 worms (CED-1::GFP) used in the first *C. elegans* experiment did not undergo RNAi treatment and were fed OP50 bacteria as a food source. Of these worms, UVC treated worms showed corpse bodies in their gonad sheath cells as well as amorphous fragmentation and damage (Figure 5). Worms treated with STS in DMSO showed corpse bodies and this result was indistinguishable from the DMSO-only treated cells. No evidence of apoptosis of was observed in the control model.

*C. elegans* MD701 worms (CED-1::GFP) used in the second *C. elegans* experiment underwent RNAi treatment. With each parameter (UVC and STS treatments) were compared between worms that were fed empty RNAi vector plasmids (pL4440:empty vector) and with pL4440:*ced-4* RNAi plasmids (Figure 6). For the *ced-4* knockdown with UVC treatment, there were no clearly observed corpse bodies, however there were the potential presence of what might be considered small corpse bodies. For the *ced-4* knockdown with STS treatment, there were no observed corpse bodies and looked similar to the negative control parameter that had *ced-4* knocked down and with no treatment. The pL4440:empty vector fed worms that were treated with DMSO-only were also observed to have corpse bodies.

## Discussion

The hypothesis of this study that UVC is a better stimulator of apoptosis than STS because UVC uses both apoptotic pathways in HeLa cells, as well as in *C. elegans*, was supported (as expected) by the results. This conclusion can be suggested from the data from the MTT cell viability assay (Figure 1) in that cell viability was substantially less

in UVC treated cells than STS. The Phalloidin and DAPI stains (Figure 2) also support this hypothesis with greater visible damage, blebbing, and amorphous morphology in UVC than STS treated cells. Figures 5 and 6 also support this hypothesis when observing the greater level of damage, blebbing, and amorphous morphology in UVC than in STS treated *C. elegans* cells.

The second hypothesis that STS will induce apoptosis via the intrinsic pathway in HeLa cells and that UVC will induce apoptosis via the extrinsic pathway in HeLa cells was supported (as expected) by the Western Blot analysis (Figure 4). Also expected, caspase 3 was cleaved by both treatments and confirm that apoptosis was induced by both treatments (Figure 4c). Similarly, both treatments cleaved and inactivated the PARP polymerase from performing any further DNA repair (an expected precursor to apoptosis induction) (Boulares *et al.* 1999). UVC treatment was the only treatment that induced caspase 8 cleavage, which is consistent with the extrinsic pathway (Figure 4a).

The final hypothesis that knocking down *ced-4* via RNAi would disrupt apoptosis in *C. elegans* was supported by the results. When comparing the empty vector UVC treatment with the *ced-4* knockdown UVC treatment, there is a distinct difference in visual appearance. The morphology of the empty vector UVC treated organism looks consistent with normal apoptotic morphology, while the knockdown model does not look like it underwent apoptosis. There are very few and very small circles around the edges that could be considered corpse bodies. If so, then this result would be consist with the alternative apoptotic pathway that utilizes DPL-1 and EFL-1/-2 to activate CED-4 and CED-3 (Schertel and Conradt 2007). This final hypothesis was also supported by the comparison of the STS treatment with RNAi knockdown which shows that no corpse bodies were formed with empty vector STS treatment that do show corpse body formation.

**Future Direction**

The result of caspase 8 not being cleaved under STS treatment (Figure 4) does not automatically confirm that the intrinsic pathway was activated. Although, concurrently, caspase 3 cleavage did occur for the STS treatment and suggests that apoptosis was induced without caspase 8 cleavage (of which it could be deduced that the intrinsic pathway was used), this result could be better verified by running an antibody stain and immunoblot for caspase 9/cleaved caspase 9. Because caspase 9 is specific to the intrinsic pathway and not to the extrinsic pathway, evidence of cleaved caspase 9 would support the suggestion that STS induces apoptosis via the intrinsic pathway. Also, by including caspase 9, it could be observed if UVC treatment induces both pathways simultaneously. Furthermore, another way to strengthen the results would be to compare the UVC and STS treatments against 12hr. and 24hr. Parameters.

An expansion of the *C. elegans* experiments would be to examine the alternative pathway to CED-4 and CED-3 core apoptotic machinery activation via DPL-1 and EFL-1/-2. A straightforward experiment could be designed to use RNAi to knockdown each of the genes for these proteins. All possible combinations would need to be observed, including each of the genes individually and both together, as well as with *ced-4* gene, and then observe if any corpse bodies persist with STS and, particularly, UVC treatment.

Furthermore, an unanswered question of whether or not *ced-4* knockdown inhibits STS apoptosis separately from DMSO-induced apoptosis could be answered if the parameters of this experiment could be improved with better establishing a DMSO concentration control model that does not induce apoptosis on its own and will allow for isolated STS apoptosis data to be produced. In this experiment, both the STS in DMSO empty vector treatment and the DMSO-only empty vector treatment induced apoptosis, thus rendering it impossible to know if STS induced apoptosis by itself without DMSO. Other studies have used higher ratio of STS to DMSO (using a higher molarity of staurosporine and a lower amount of DMSO to better isolate the STS treatment (2003). Another explanation for this would be to verify that the treated worms were not older adults and thus producing corpse bodies. A recommended future experiment would have verified L4 worms for treatment and then imaging as Day 1 adults.

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# Appendix

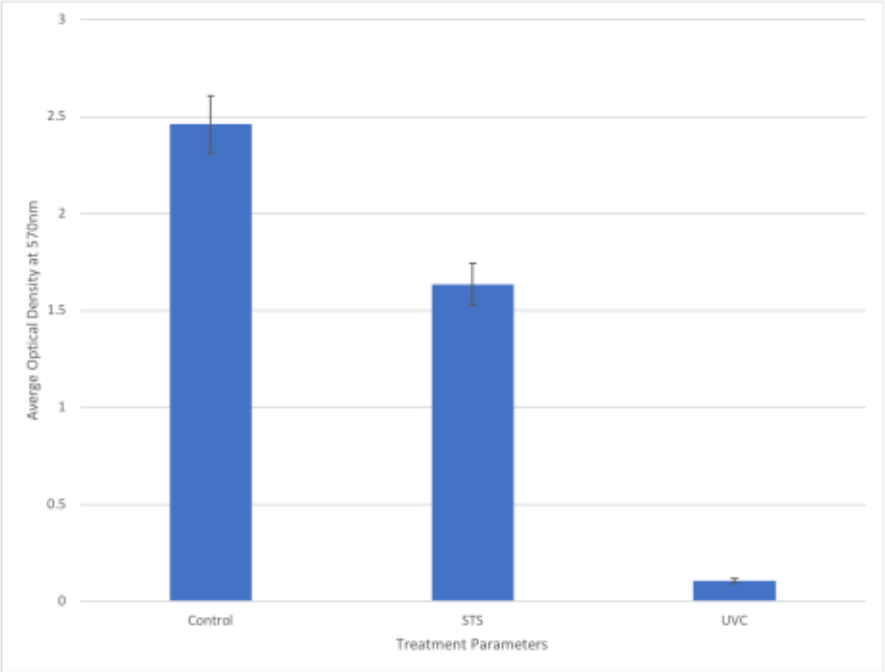


**Figure 1 | Average optical density of MTT cell viability assay.** This graph is a representation of mitochondrial activity using MTT cell viability assay comparing treatment parameters with HeLa cells. This graph shows the optical densities as measured by spectrophotometry at 570nm and represents the amount of MTT formazan produced by viable cells. Cells treated with UVC show a substantial decrease in cell viability compared to the control and STS parameters. Cells treated with STS show an decrease from the control. *T* test results: Control vs. UVC: two-tailed *P* value <0.0001 (extremely statistically significant); Control vs. STS: two-tailed P value <0.0001 (extremely statistically significant); Control vs. STS: two-tailed *P* value = 0.0012 (very statistically significant).

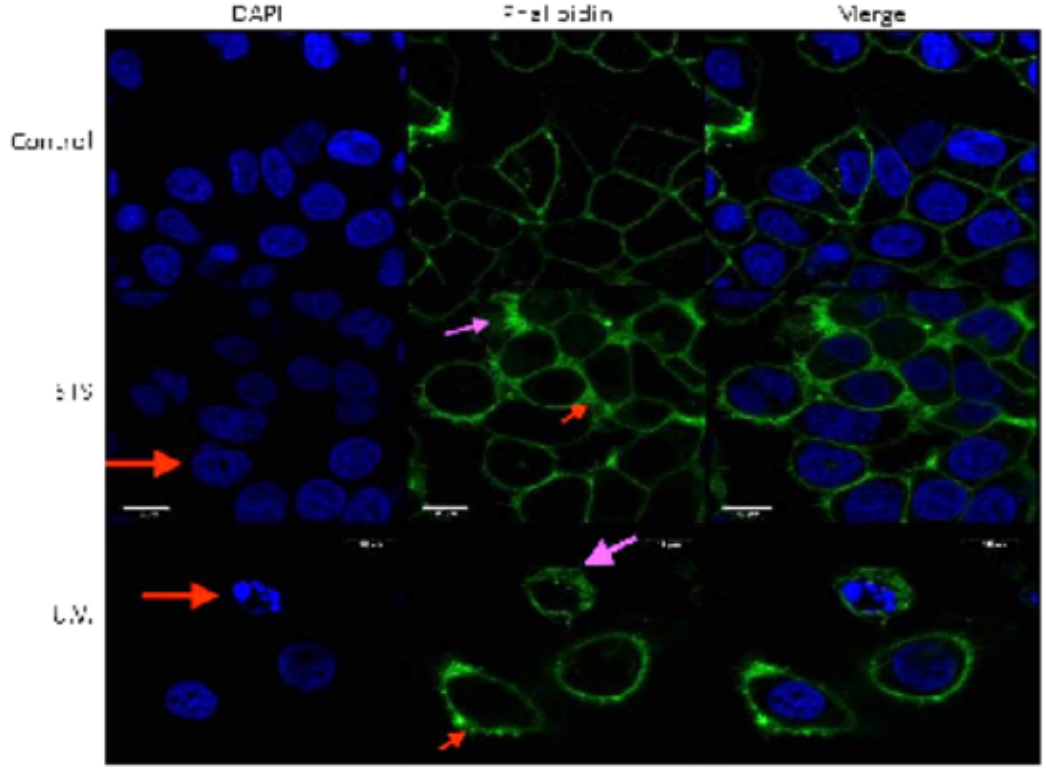


**Figure 2 | Phalloidin and DAPI stains.** This image shows the morphological images of UVC and STS treatments of HeLa cells. For the control, normal morphology is present for both the nucleus (DAPI stain) and for Actin (Phalloidin stain). For UVC treated cells, the DAPI image show fragmented and misshapen nuclear envelopes (red arrow), and the Phalloidin image shows actin filaments that are blebbing (red arrow) and breaking down (purple arrow). For STS treated cells, the DAPI image shows breakdown of the nuclear envelope (red arrow) and the Phalloidin image shows blebbing (red arrow) and breakdown (purple arrow).

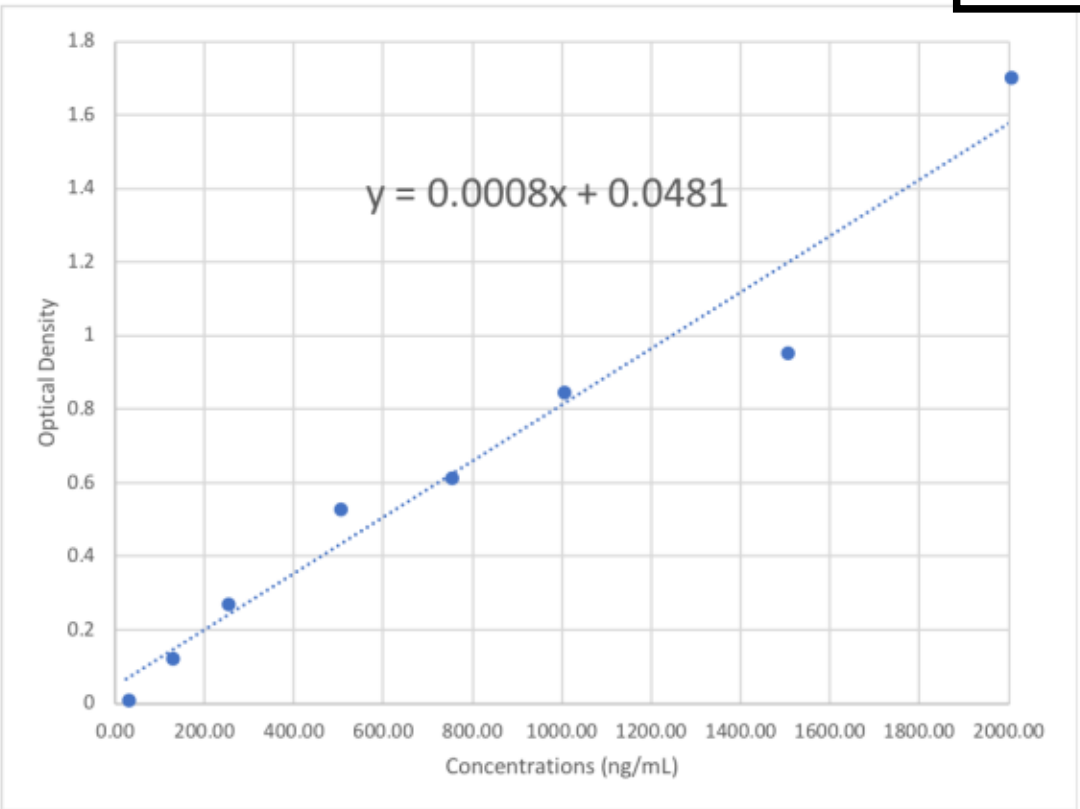


**Figure 3 | BCA Assay Standard Curve.** The equation for the standard curve was found to be y = 0.0008x + 0.0481 and was used to calculate the standardized protein concentrations (ng/mL) with x = optical density and y = concentration. Standardized protein concentration was found to be 107.11ng/mL.

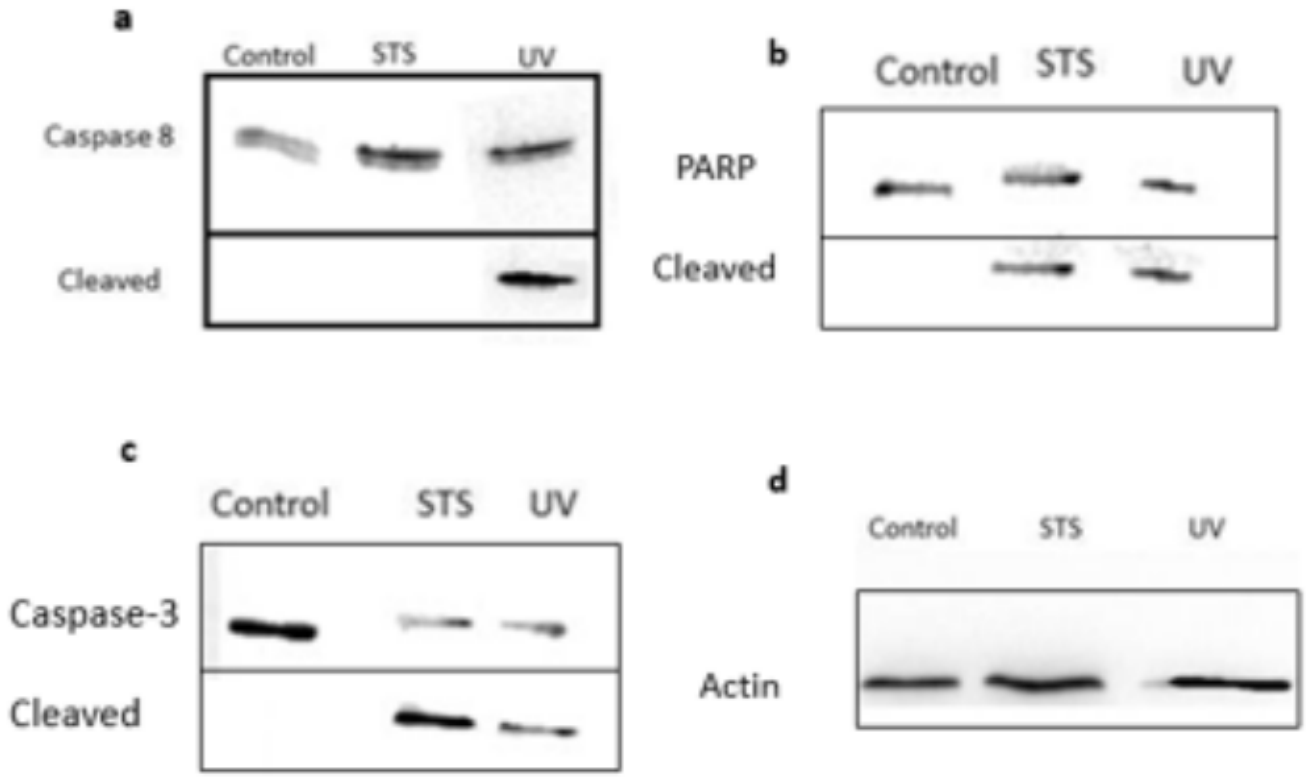


**Figure 4 | Western Blot Apoptosis Analysis. a.** Cleaved caspase-8 was not observed in the control or STS treated HeLa cells, but only in the UVC treated cells. **b.** Cleaved PARP was observed in UVC and STS treatments. **c.** Cleaved caspase-3 was observed in UVC and STS treatments. **d:** Actin is the control and was verified.

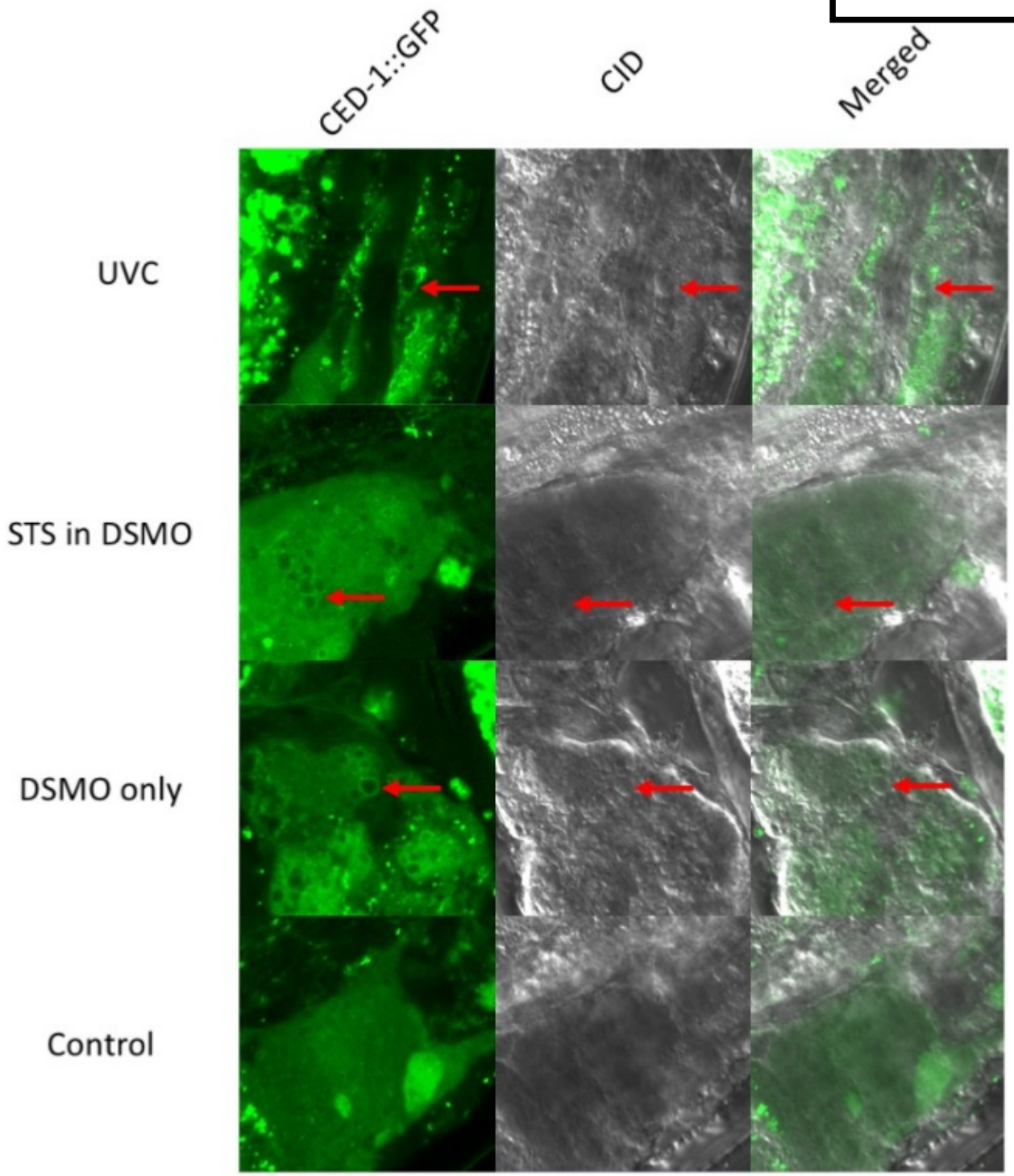


**Figure 5 | *C. elegans* MD701 experiment representative images visualized using CED-1::GFP.** UVC treated cells show corpse bodies (red arrows) and amorphous fragmentation of cellular contents. STS in DMSO and DMSO only treated cells both show corpse bodies. Control cells show no corpse bodies and intact cellular environment.

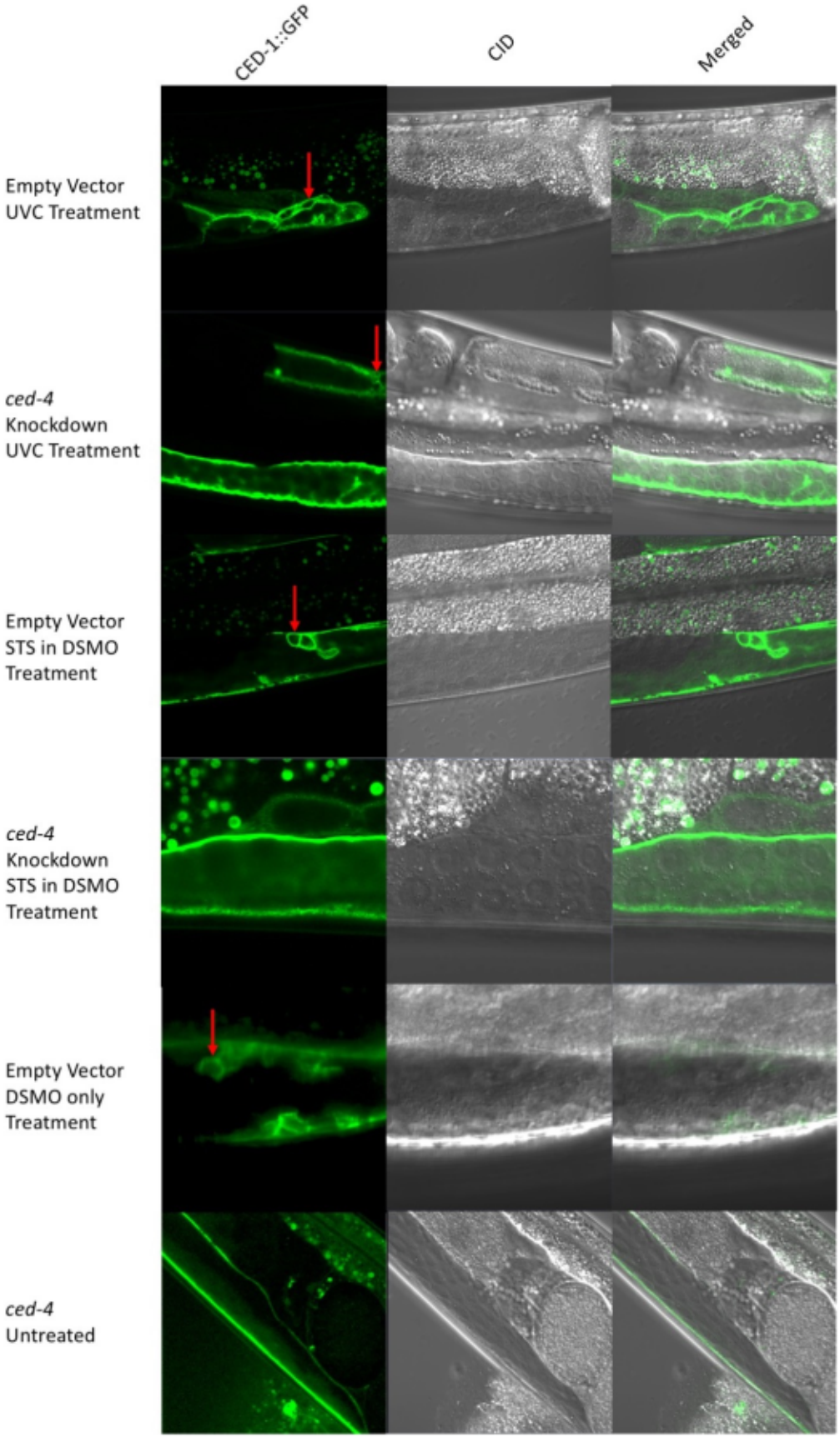


**Figure 6 | *C. elegans* MD701 experiment representative images visualized using CED-1::GFP and RNAi bacterio.** With each parameter compared between empty vector (UVC, STS, and DMSO) and the *ced-4* knockdowns, there were little to no observed corpse bodies (red arrows) with the respective knockdowns.