Aurigene Pharmaceutical Services

Evaluate Gunalight mediated modulation of cellular effects (ATP, ROS) in HaCaT and HEK293

22nd May 2026

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Evaluation of ATP levels in HaCaT cells post exposure to Gunalight

Objective:

- The objective is to evaluate the changes in ATP and ROS levels post exposure of cells to 1h of Gunalight followed by incubation in dark for either 16h or 24 h

Protocol

0.1×10^6 cells/mL were seeded in a 24-well plate

37°C, 5% CO_2 for 24 h

Spent culture media was replaced with 200 µL Fluorobrite DMEM media

Cells were exposed to light for 60 minutes and subsequently incubated for 16 and 24 hours in dark. Control assay plates, unexposed to Gunalight, were maintained in the dark under identical incubation conditions

CTG reagent (100 µL) was added to 100 µL of cell-containing media for both light-exposed and unexposed cells. For H_2O_2 treatment, cells were exposed to 2 mM H_2O_2 for 15 minutes, followed by the addition of 100 µL of CTG reagent

Assay plate was incubated at room temperature in the dark for 12 minutes, 300 rpm to allow the luminescent signal to stabilize

A luminescence endpoint protocol was measured using Cytation 5 multimode plate reader with an integration time of 1 second per well

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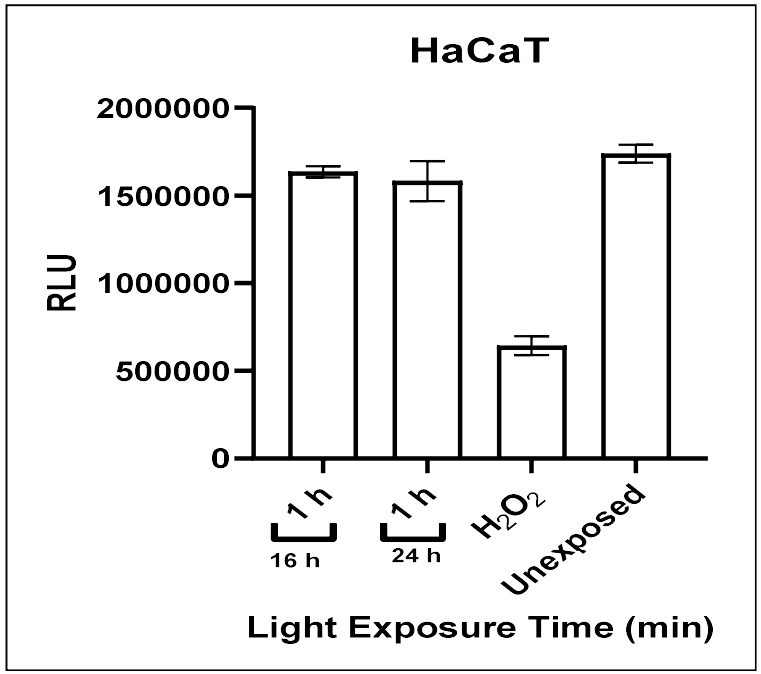
Evaluation of ATP levels in HaCaT cells post exposure to Gunalight



Conclusion:

- No changes in ATP levels were observed in HaCaT cells incubated for either 16 h or 24 h post 1h light exposure
- As expected, reduced ATP levels were observed for cells treated with H_2O_2 (+ve control well) as compared to unexposed cells because of peroxide shock

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Evaluation of changes in Intracellular and Mitochondrial ROS levels in HaCaT cells post exposure to Gunalight

Objective: The objective is to evaluate the changes in Intracellular and Mitochondrial ROS levels in HaCaT cells post exposure to Gunalight

Experiment: Post-Stain Protocol

0.1 x 10^6 cells/mL were seeded in a 24-well plate

37°C, 5% CO_2 for 24 h

Cell culture media was removed, and cells were washed (1x) with HBSS buffer

Cells were exposed to light for 60 minutes in 200 µL of Fluorobrite DMEM, while unexposed plate was maintained in the dark as a control

37°C, 5% CO_2 for 16 & 24 h in dark

Cells were stained with 20 µM H2DCFDA, 1 µM MitoSox, 1: 10,000 Hoechst stain prepared in Fluorobrite DMEM media

37°C, 5% CO_2 for 35 min

Cells were washed (2x) with prewarm HBSS buffer and 200 µL of Fluorobrite DMEM media was added to each well post washing

Hydrogen peroxide at a concentration of 2 mM was added to the control wells and incubated for 15 min at 37°C, 5% CO_2

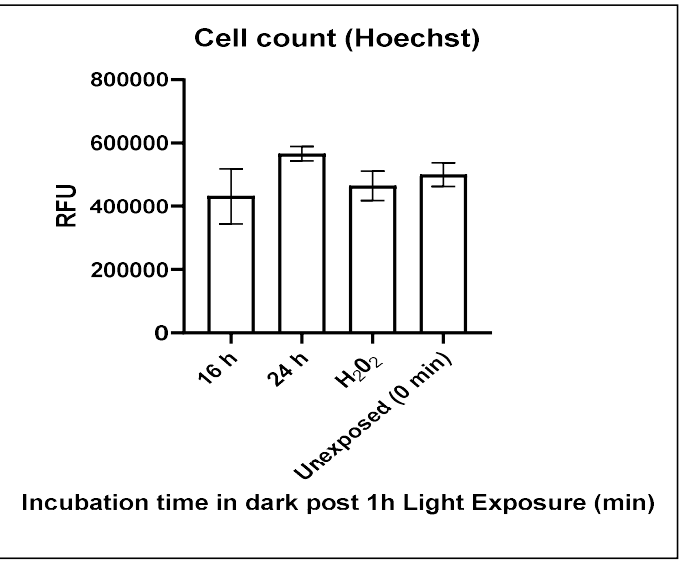
All data (Fluorescence read and imaging) were acquired using a Cytation 5 Cell Imaging Multi-Mode Reader

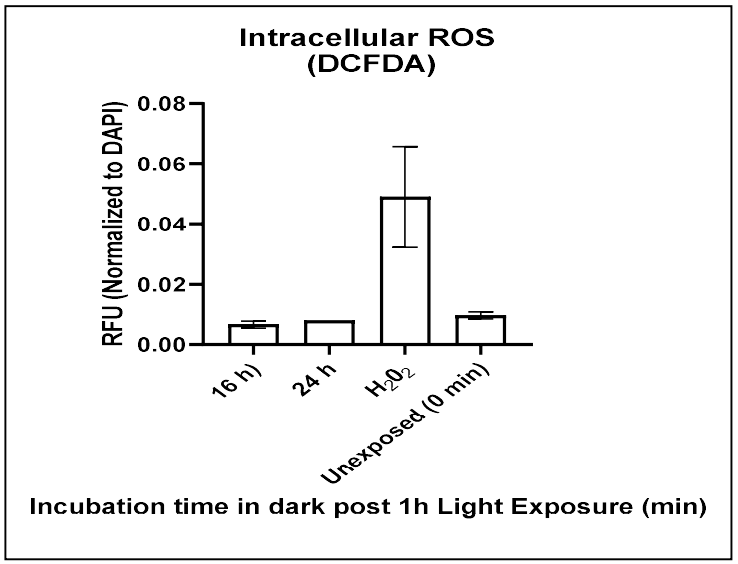
Samples were imaged using a 10x Olympus objective

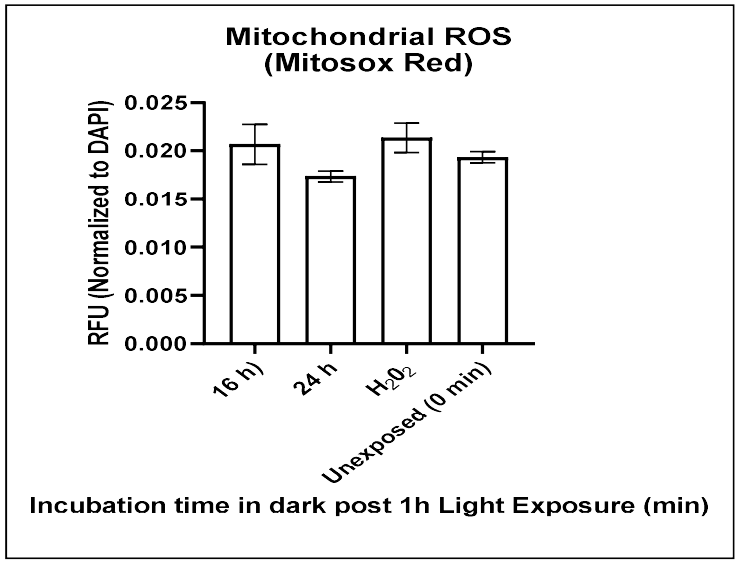
Samples were imaged using brightfield and three fluorescence channels (DAPI, GFP, and Texas Red) using the respective LED filter cubes

Wavelengths : Ex 350 nm, Em 461 nm for Hoechst (DAPI); Ex 396 nm, Em 580 nm for Mitosox(Texas Red); Ex 485 nm , Em 530 nm for H2DCFDA (GFP)

Evaluation of changes in Intracellular and Mitochondrial ROS levels in HaCaT cells post exposure to Gunalight (Fluorescence reads)







Conclusion:

- No modulation in intracellular ROS levels was observed in cells exposed to Gunalight for 1 hour and subsequent incubation in the dark for either 16 h or 24 h

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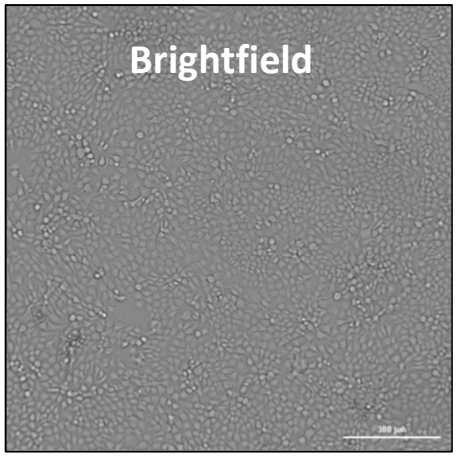
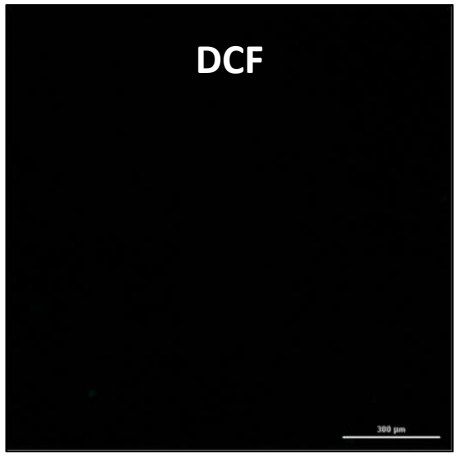
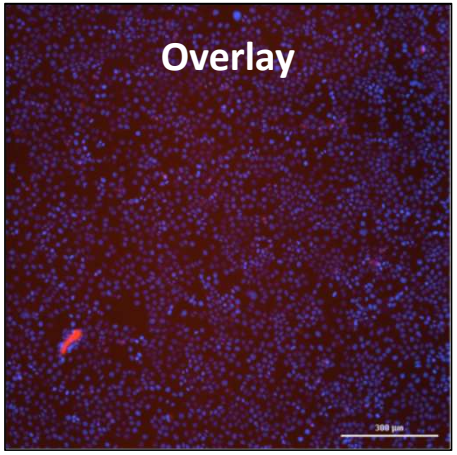
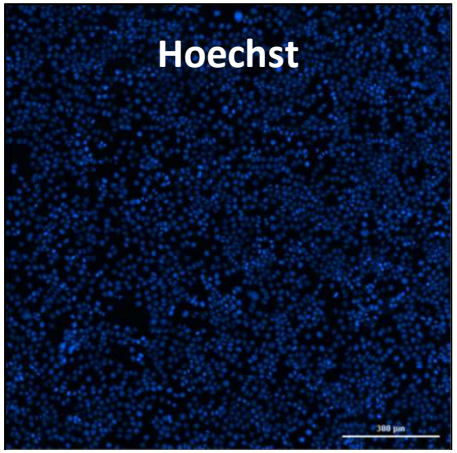
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HaCaT cells – control wells - Unexposed (Images)

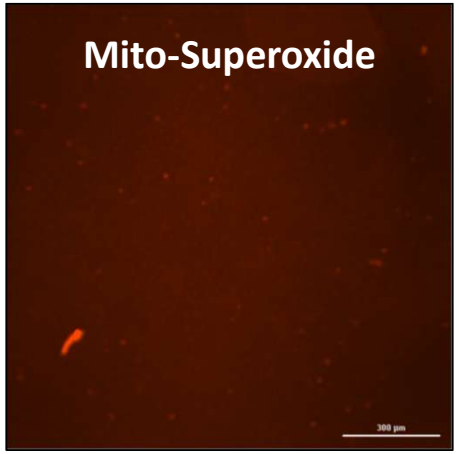
Light exposure:
Unexposed (0 min)











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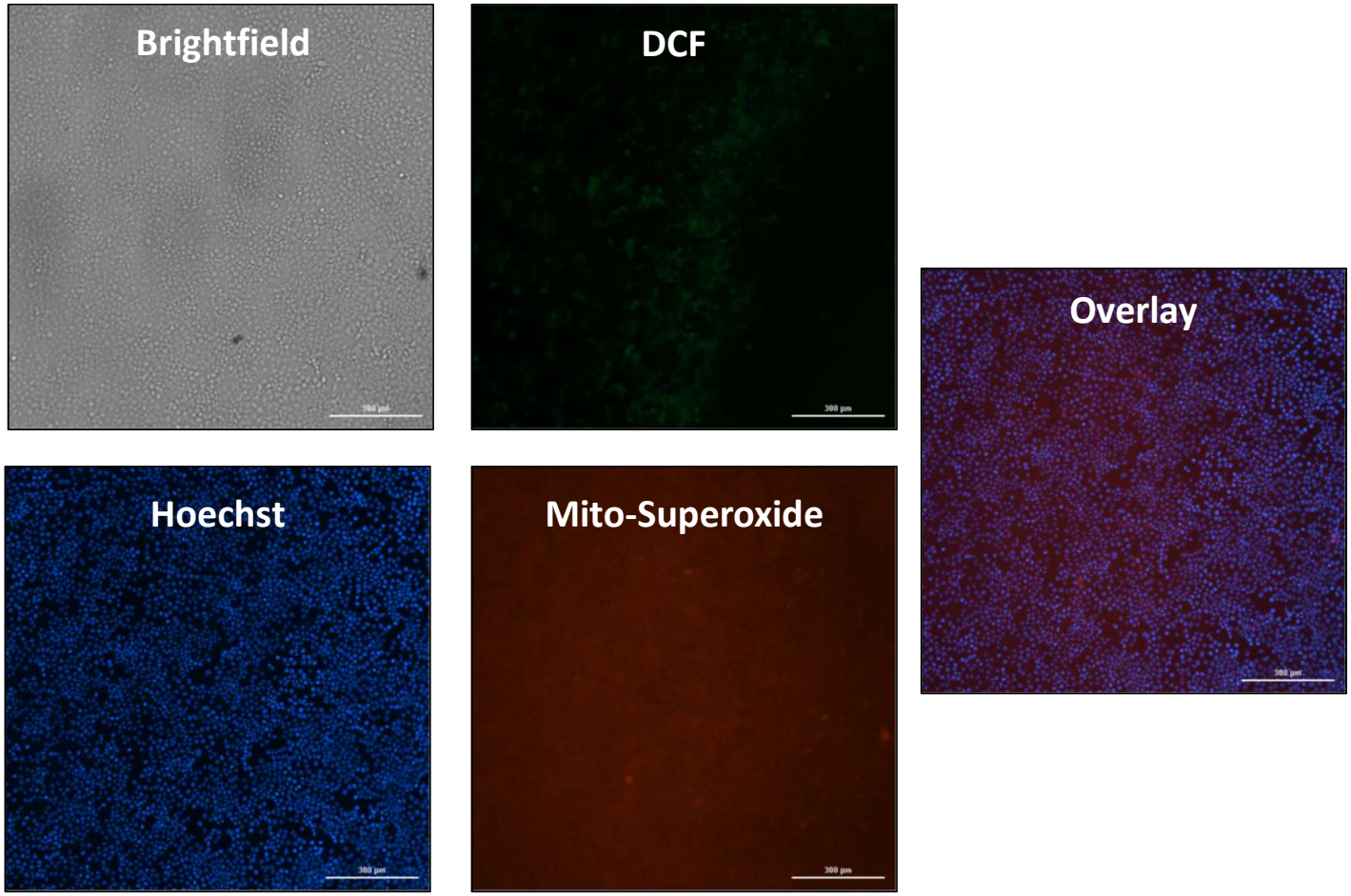
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HaCaT cells post exposure to H_2O_2 (Images)

Light exposure:

Control -

H_2O_2 treated



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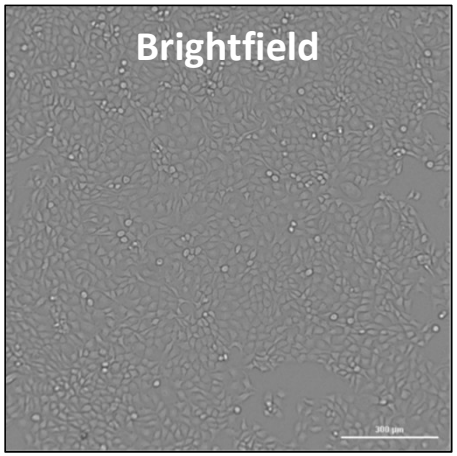
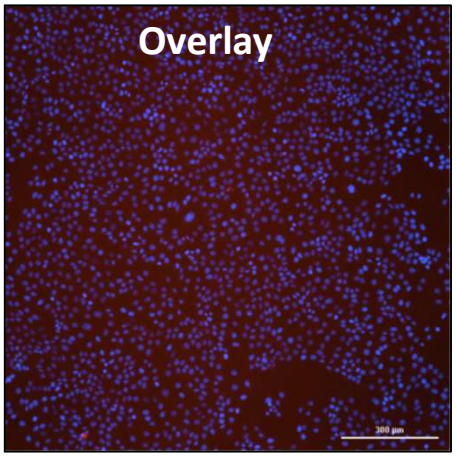
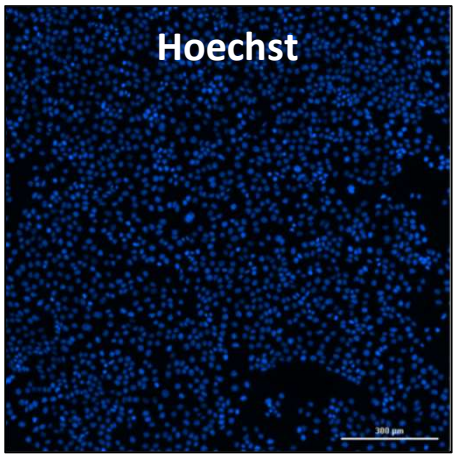
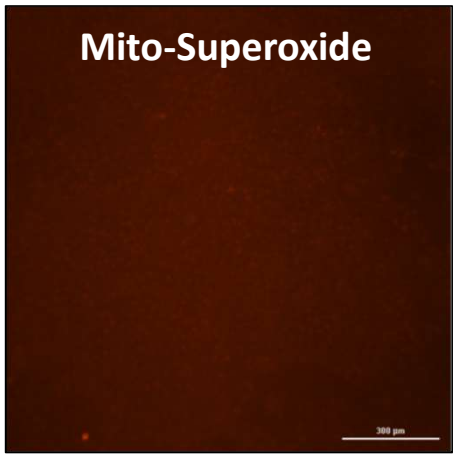
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HaCaT cells post exposure to Gunalight for 1 h followed by incubation in dark (Images)

Light exposure:
1 h
Incubation in dark:
16 h









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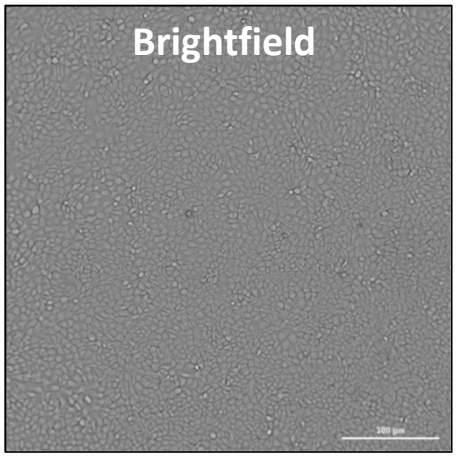
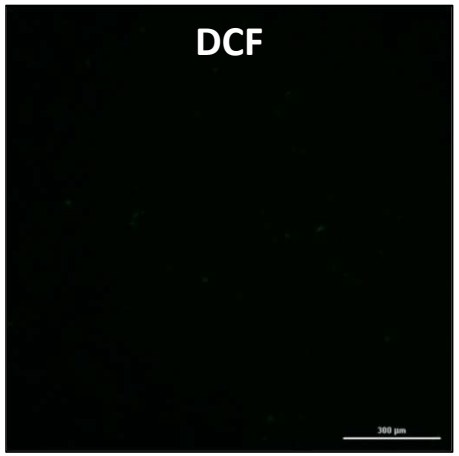
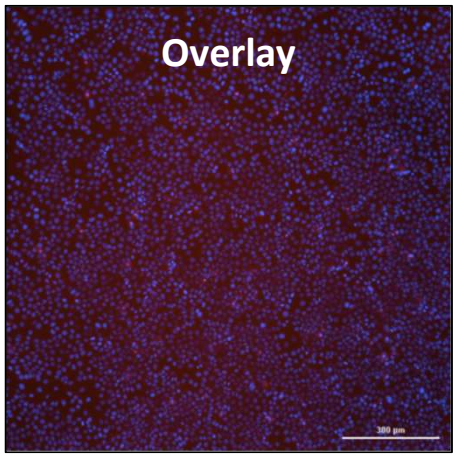
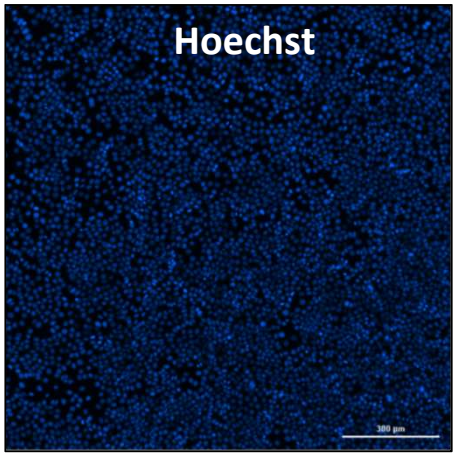
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HaCaT cells post exposure to Gunalight for 1 h followed by incubation in dark (Images)

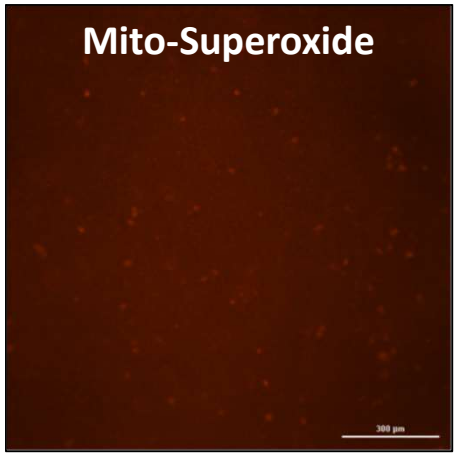
Light exposure:
1 h
Incubation in dark:
24 h











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Evaluation of ATP levels in HEK293 cells post exposure to Gunalight

Protocol

0.1×10^6 cells/mL were seeded in a 24-well plate

37°C, 5% CO_2 for 24 h

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Cells were exposed to light for 60 minutes and subsequently incubated in dark for 16 and 24 hours. Control assay plates, unexposed to Gunalight, were maintained in the dark under identical incubation conditions

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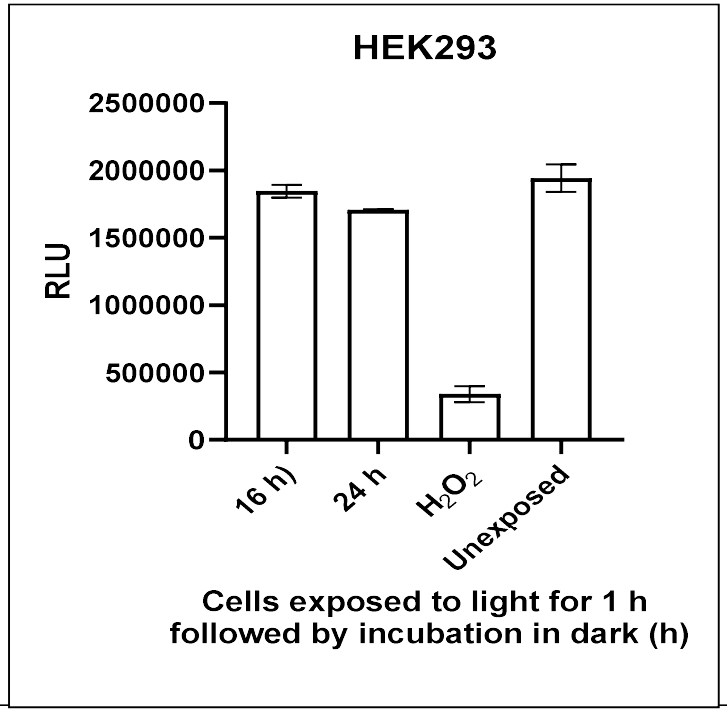
Evaluation of ATP levels in HEK293 cells post exposure to Gunalight



Conclusion:

- No significant changes in ATP levels were observed in HEK293 cells exposed to Gunalight for 1h followed by incubation in dark for 16 h and 24 h
- As expected, reduced ATP levels were observed for cells treated with H_2O_2 (+ve control well) as compared to unexposed cells because of peroxide shock

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Evaluation of changes in Intracellular and Mitochondrial ROS levels in HEK293 cells post exposure to Gunalight

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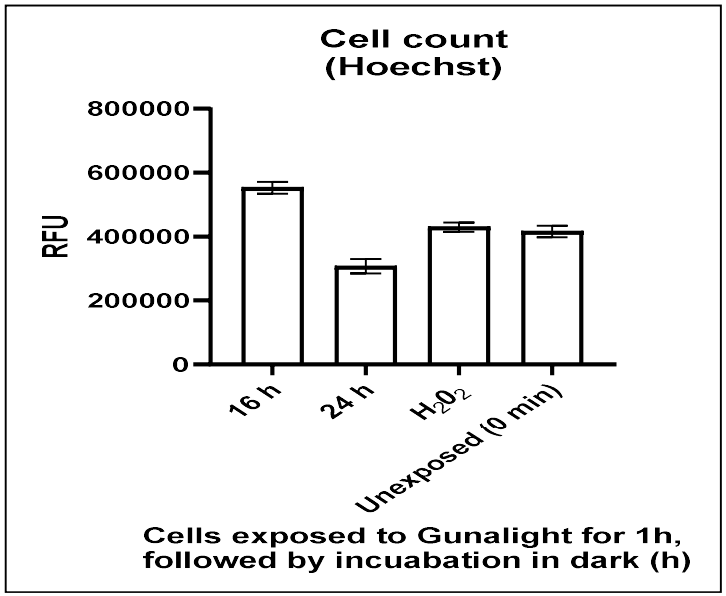
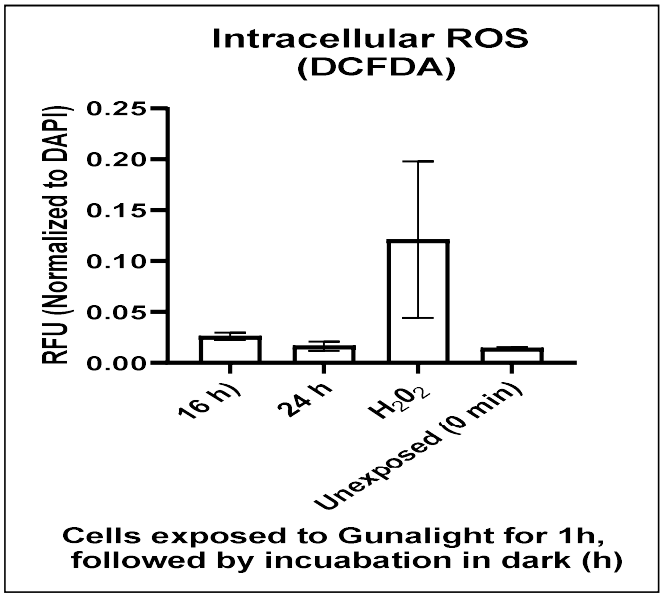
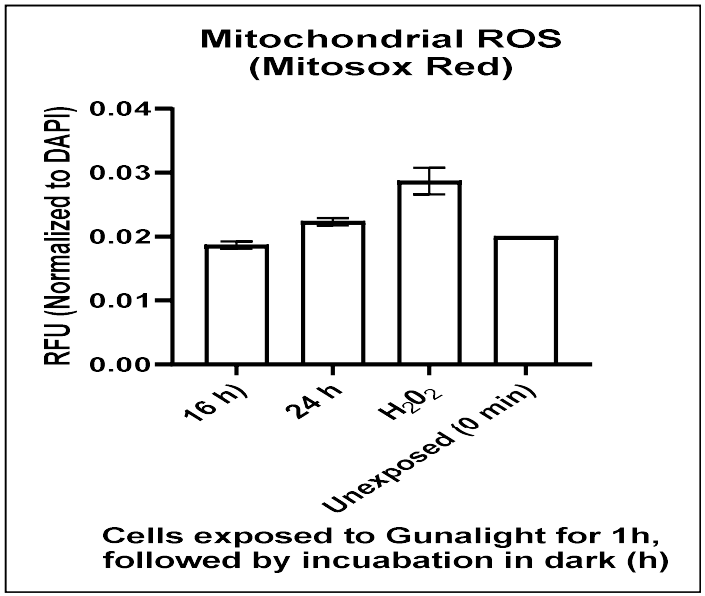
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HEK293 cells post exposure to Gunalight (Fluorescence reads)







Conclusion:

- No significant changes in Mitochondrial and Intracellular ROS levels were observed for HEK293 cells incubated in dark for 16 h and 24 h post Gunalight exposure of 1 h over unexposed control

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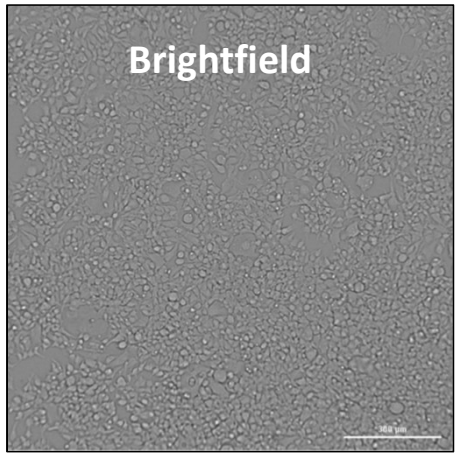
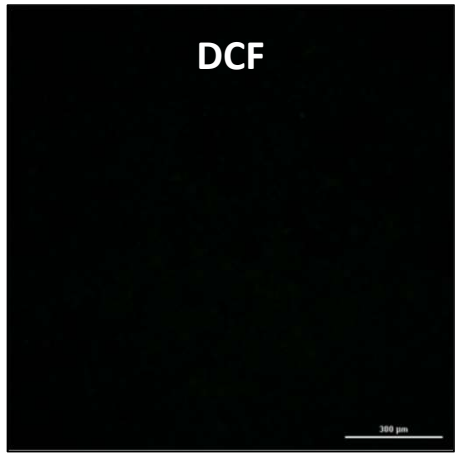
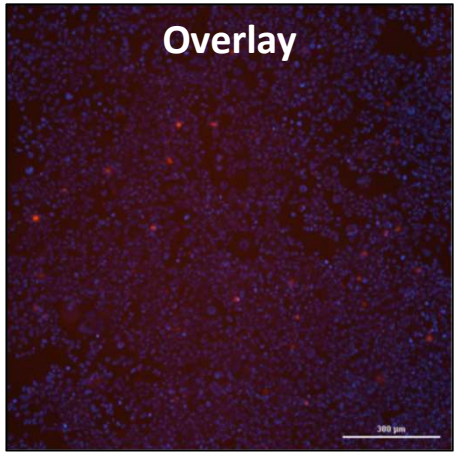
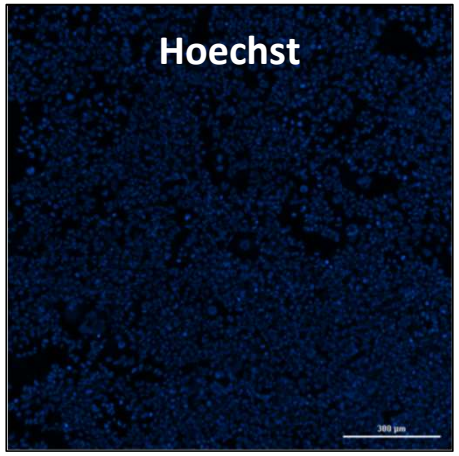
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HEK293 cells – control wells - Unexposed (Images)

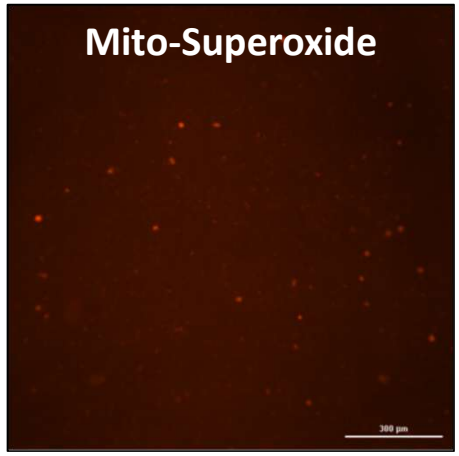
Light exposure:
Unexposed (0 min)











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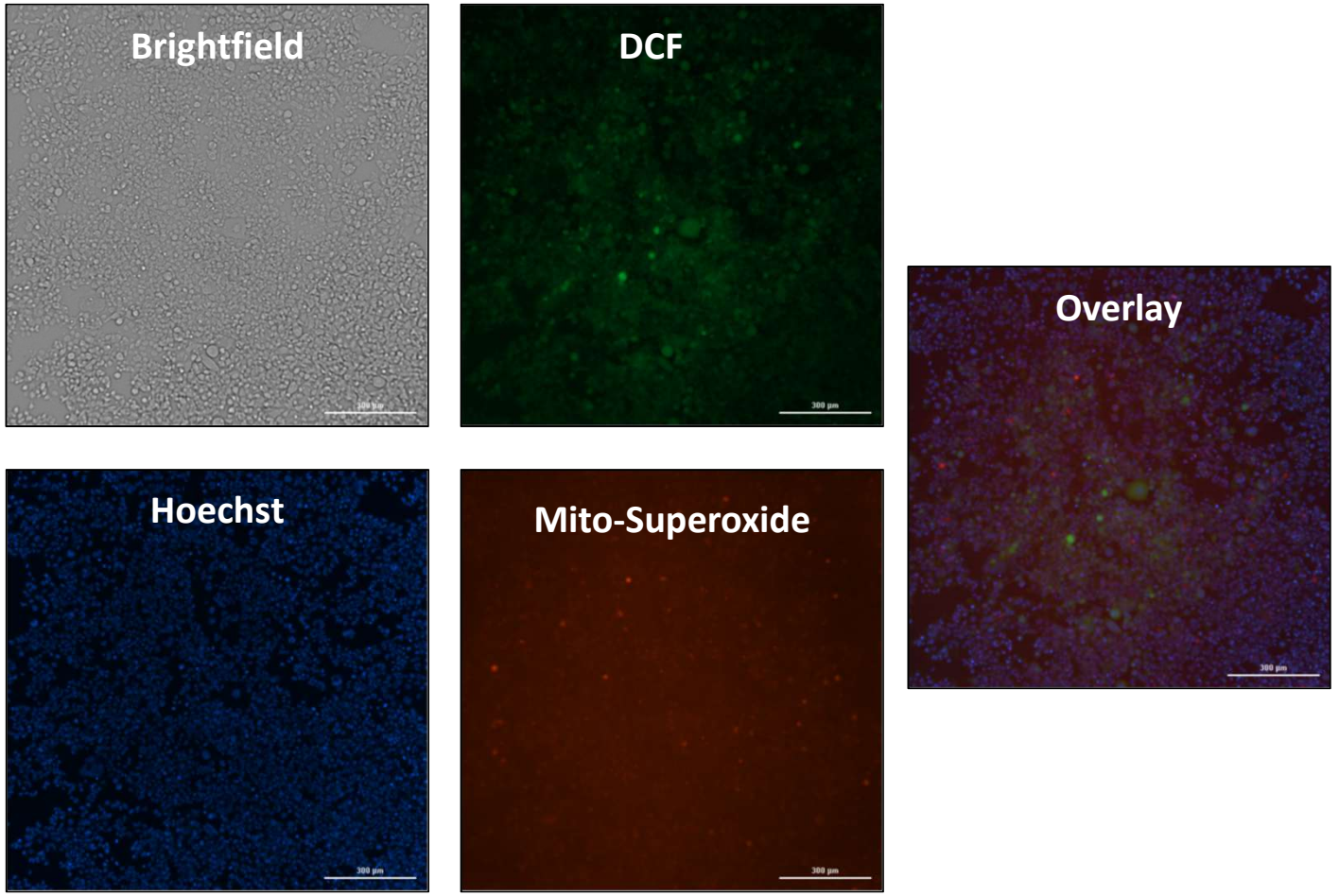
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HEK293 cells exposed to H_2O_2 (Images)

Light exposure:
Control cells -
H_2O_2 treated



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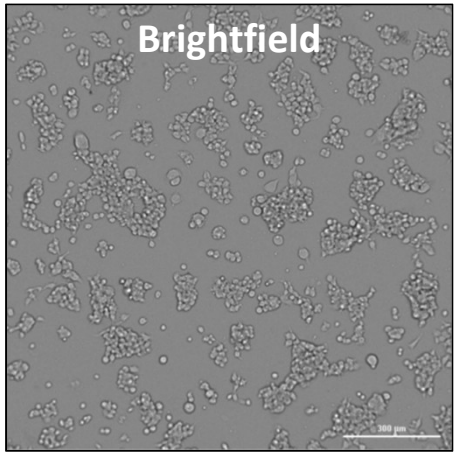
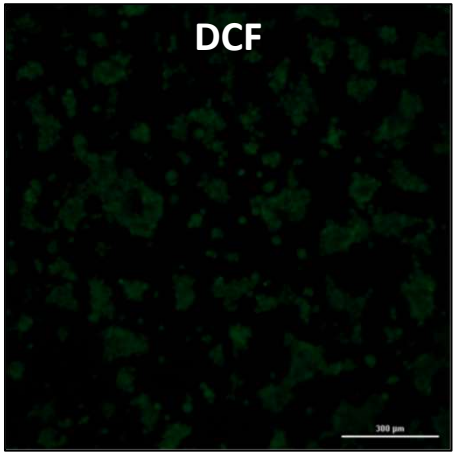
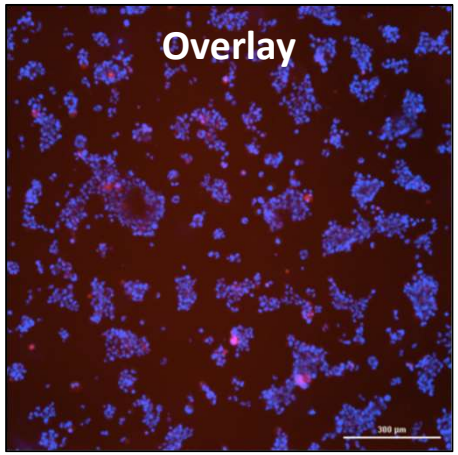
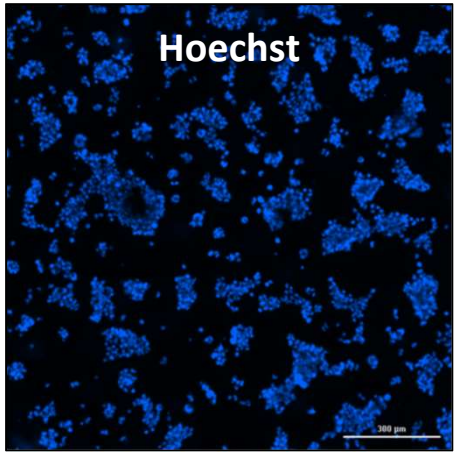
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HEK293 cells post exposure to Gunalight for 1 h followed by incubation in dark (Images)

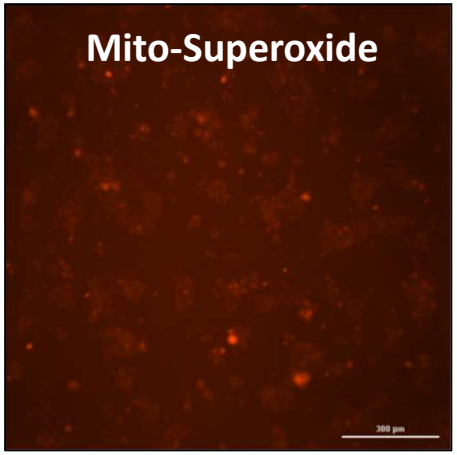
Light exposure:
1 h
Incubation in dark:
16 h











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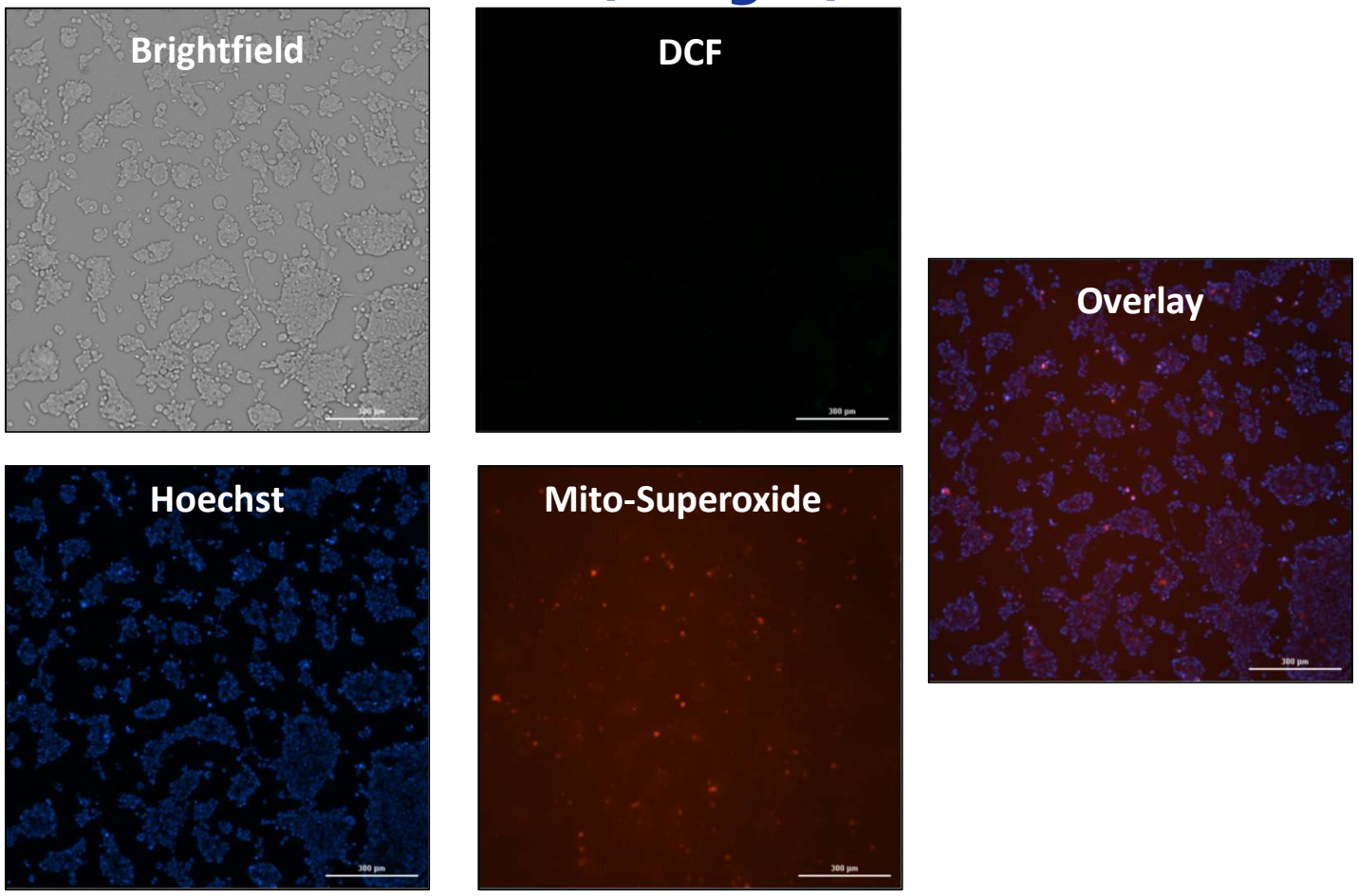
Evaluation of changes in Intracellular and Mitochondrial ROS levels in HEK293 cells post exposure to Gunalight for 1 h followed by incubation in dark (Images)

Light exposure:
1 h
Incubation in dark:
24 h



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Summary

- ATP levels and Fluorescence intensities for both DCFDA (Total ROS) and MitoSOX (mitochindrial ROS) remained comparable to the unexposed controls, no elevation in ATP levels, intracellular and mitochondrial ROS levels were observed following 1 hour exposure of Gunalight and subsequent incubation in dark for 16 and 24 hour

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Thank You