**REGISTRY**

**WP4 Effects of combined SCR and UV radiation fields on biological systems**

# DETAILED LIST OF BIOLOGICAL SAMPLES, MAJOR PROTOCOLS AND REAGENTS USED IN ALL EXPERIMENTS UNDER WP4 21GRD02 BIOSPHERE PROJECT (PERIOD 2023-2024)

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## Cells and reagents

Cells:

- Human keratinocytes HaCaT (HaCaT cells, Cat no.: 300493, CLS Cell Lines Service, Eppelheim, Germany)
- Human skin fibroblasts Hs27 (ATCC, Hs27 CRL-1634™, Manassas, VA, USA)
- Human peripheral blood monocytes (CRL 9855, US Pat. No. 5.447.861, ATCC, Manassas, VA, USA).
- Human peripheral blood lymphocytes (2 Donors, C. Beinke **BIR**, DE)

Cell culture:

- Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies, Burlington, Canada)
- Fetal bovine serum (SFB) (PAN Biotech GmbH, Aidenbach, Germany)
- Antibiotic-antimycotic solution (100X) (Sigma-Aldrich, Saint Louis, USA)
- Iscove's Modified Dulbecco's Medium (IMDM) culture medium (Life Technologies, Burlington, Canada)
- HT supplement (Gibco™ HT Supplement, Life Technologies, Burlington, Canada)
- Phosphate buffered saline without MgCl2 and CaCl2 (DPBS, Dulbecco's phosphate buffered saline, Sigma, Saint Louis, USA)
- Trypsin/EDTA 0.05%/0.02% (PAN Biotech GmbH, Aidenbach, Germany)
- Trypan blue (Trypan Blue Solution, 0.4%, Gibco™, Life Technologies, Burlington, Canada)

MTS reduction:

- CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay kit (Promega Corporation, Madison, USA)

Lactate dehydrogenase (LDH) release:

- CytoTox 96® Non-Radioactive Cytotoxicity Assay kit (Promega Corporation, Madison, USA)

Gene expression:

- RiboZol™ RNA Extraction Reagent (N580 Avantor, VWR Funding, Radnor, Pennsylvania, USA)
- High Capacity cDNA Reverse Transcription (Applied Biosystems, Waltham, SUA)
- $RT^2$ First Strand Kit (330404, Qiagen, Hilden, Germany)
- $RT^2$ SYBR ® Green/ROX qPCR Mastermix (330523, Qiagen, Hilden, Germany)
- $RT^2$ Profiler PCR Array ($RT^2$ Profiler™ PCR Array Human Stress & Toxicity PathwayFinder, GeneGlobe ID-PAHS-003Z, Qiagen, Hilden, Germany)

**Protocols**

*Preparation of cells for exposure to protons and UVB:*

Adherent HaCaT and Hs27 cells were detached with Trypsin/EDTA 0.05%/0.02% (P10-023100, PAN Biotech GmbH, Aidenbach, Germany). All the cell suspensions (HaCaT, Hs27 and CRL 9855) were centrifuged and suspended in RPMI 1640 culture medium w/o phenol red (11835030, Gibco, Life Technologies, Burlington, Canada), supplemented with 10% FBS. Cells were counted using a Burker-Turk counting chamber, and viability was assessed by the trypan blue exclusion test. Finally, cells were suspended in RPMI 1640 culture medium without phenol red at a cell density of 0.7 -1 x $10^7$ cells/mL for HaCaT or Hs27 cells, and $10^7$ cells/mL for CRL 9855 monocytes. Finally, 100 µL of cell suspension were placed in a well of a low adhesion 48 well plate (677102, Cellstar, Greiner Bio One, Kremsmünster, Austria) or in the dish (provided by PTB), for proton exposure top-to-bottom or bottom-to-top, respectively. Distinct samples were prepared for UVB or proton exposure, as well as samples for co-exposure to protons and UVB. Various non-irradiated controls, containing cells that were handled like the irradiated ones, were used. The irradiation control was kept in the irradiation room, outside the proton beam, while the lab control was kept in the biology laboratory, outside the irradiation room. Co-exposed samples were first irradiated with protons and, within 20 min thereafter, to UVB. Cells were placed in clear low adhesion 48 well plates for UVB exposure. Thus, cells exposed to protons in low adhesion 48 well plates were directly transferred to UVB exposure, while cells placed in dishes for proton exposure were first transferred to low adhesion 48 well plates, and then exposed to UVB.

*Cellular viability assays:*

The viability of cells exposed to protons and UVB was evaluated by the MTS [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)] reduction test, which provides information on the number of metabolically active cells in culture, complemented with the lactate dehydrogenase (LDH) release assay, which offers information about the alteration of the plasma membrane integrity in relation with necrotic cell death. The kits used were the CellTiter 96® AQueous One Solution Cell Proliferation Assay and the CytoTox 96® Non-Radioactive Cytotoxicity Assay from Promega Corporation (Madison, WI, USA), respectively.

Cells were cultivated in triplicates in 96 well plates (5 x $10^3$ HaCaT keratinocytes and 10 x $10^3$ Hs27 fibroblasts in 100 µL complete culture medium) for 24 h and 48 h at 37ºC, in 5% CO2 atmosphere.

For the LDH release assay, 50 µL of supernatant were collected from each sample, and were transferred to a 96 well plate. 50 µL of LDH substrate were added to supernatants, and samples were incubated for 30 min at room temperature in the dark. The reaction was stopped with the solution contained in the kit. The colorimetric reaction was measured as described below.

For the MTS reduction test, 50 µL complete culture medium were added in the original culture plate to restore the 100 µL total volume of the culture. In each sample, 20 µL MTS were added,

and cells were incubated for 90 min at 37ºC, in 5% CO2 atmosphere. The colorimetric reaction was measured as described below.

Colorimetric reactions were measured using a Tecan ELISA reader (Männedorf, Switzerland) at 490 nm against a 620 nm reference wavelength for the MTS reduction test, and at 490 nm for the LDH release test. Optical density (OD) data were acquired and processed using the Magellan software (Tecan, Männedorf, Switzerland). The OD values in experimental and control samples were corrected by subtracting the mean OD of background samples. Results were presented as mean corrected OD ± standard error of the mean (SEM) for triplicate samples.

*Gene expression:*

Irradiated and non-irradiated controls of HaCaT and Hs27 cells ($0.5 \times 10^6$ /sample) were cultivated for 24-48 h in 6 well plates in a total complete medium volume of 2.5 mL, while CRL 9855 monocytes ($0.9 \times 10^6$ cells) were cultivated for 24 h in 24 well plates in 2 mL complete medium. At the end of the cultivation time, the supernatant of the HaCaT and Hs27 cultures was discarded, the adherent cells, presumed to be viable cells, were washed with Dulbecco's phosphate buffered saline (DPBS, D8537, Sigma-Aldrich, Saint Louis, USA), and were finally suspended in 1 mL RiboZol™ RNA Extraction Reagent (N580 Avantor, VWR Funding, Radnor, Pennsylvania, USA). Non-adherent CRL 9855 monocytes were washed by centrifugation with DPBS, and were finally suspended in RiboZol™ RNA Extraction Reagent. All samples in RiboZol were stored frozen at around -18°C at PTB for around 7 days at most, being thereafter transported in dry ice to IVB, where samples were stored at -80°C until use.

Gene expression was evaluated by qRT-PCR, as previously described [1]. Total concentration of RNA in RiboZol samples was measured using the Nanodrop 2000 equipment (NanoDrop Technologies, Wilmington, DE, USA), the registered values of the 260/280 nm and 260/230 nm ratios being above 1.8. The $RT^2$ First Strand Kit (Qiagen, Hilden, Germany) was used for cDNA synthesis. The expression of 84 genes involved in stress and toxicity was evaluated using the $RT^2$ Profiler™ PCR Array Human Stress & Toxicity PathwayFinder (PAHS-003Z, Qiagen, Hilden, Germany) (Table 1), and the SYBR Green chemistry on ABI7500 Fast PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The expression level of each gene was normalized to the geometric mean value of housekeeping genes that were selected according to RefFinder algorithm [2]out of five candidate reference genes (ACTB, B2M, GAPDH, HPRT1 and RPLP0). Gene expression data were analyzed with the $RT^2$ Profiler PCR Array software package (Qiagen, Hilden, Germany). Gene expression levels were calculated as $2^{-\Delta CT}$ values. Fold Change (FC) in gene expression was calculated as the $2^{-\Delta CT}$ values in the irradiated sample divided by the $2^{-\Delta CT}$ value in control. FC values > 1 designate gene over-expression, while FC values < 1 designate gene under-expression.

**Table 1**. Stress genes in the RT² Profiler™ PCR Array Human Stress & Toxicity PathwayFinder (PAHS-003Z, Qiagen).

| | | |
|---|---|---|
| **Cell Death** | ***Apoptosis***<br>CASP1 (ICE), FAS, MCL1, TNFRSF10A (TRAIL-R), TNFRSF10B (DR5), TNFRSF1A (TNFR1). | |
| | ***Necrosis***<br>FAS, GRB2, PARP1 (ADPRT1), PVR, RIPK1, TNFRSF10A (TRAIL-R), TNFRSF1A (TNFR1), TXNL4B. | |
| | **Autophagy**<br>ATG12, ATG5, ATG7, BECN1, FAS, ULK1. | |
| **DNA Damage & Repair** | ***Cell Cycle Arrest & Checkpoints***<br>CDKN1A (p21CIP1, WAF1), CHEK1, CHEK2 (RAD53), DDIT3 (GADD153, CHOP), HUS1, MRE11, NBN, RAD17, RAD9A. | |
| | ***Other DNA Damage Responses***<br>ATM, ATR, DDB2, GADD45A, GADD45G, RAD51, TP53 (p53), XPC. | |
| **Unfolded Protein Response** | ATF4, ATF6, ATF6B, BBC3 (PUMA), BID, CALR, DDIT3 (GADD153, CHOP), DNAJC3, HSP90AA1, HSP90B1, HSPA4 (HSP70), HSPA5 (GRP78). | |
| **Oxidative Stress**<br>FTH1, GCLC, GCLM, GSR, GSTP1, HMOX1, NQO1, PRDX1, SQSTM1, TXN, TXNRD1. | | **Hypoxia Signaling**<br>ADM, ARNT, BNIP3L, CA9, EPO, HMOX1, LDHA, MMP9, SERPINE1 (PAI-1), SLC2A1, VEGFA. |
| **Inflammatory Response**<br>CCL2 (MCP-1), CD40LG, CRP, CXCL8 (IL8), IFNG, IL1A, IL1B, IL6, TLR4, TNF. | | **Osmotic Stress**<br>AKR1B1, AQP1, AQP2, AQP4, CFTR, EDN1, HSPA4L (OSP94), NFAT5, SLC5A3. |

References

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*DNA Damage γH2AX Immunofluorescence assay for measurement of DNA damage*

Human Fibroblasts HS-27 and Human Keratinocytes HaCaT: Exposed and control cells in suspension were plated over 22x22 $mm^2$ coverslips in 35 mm dishes, and were harvested at various time points (4 and 24 h) for the γH2AX assay. Cells were fixed for 15 min at room temperature (RT) with paraformaldehyde 3% (F8775, Sigma-Aldrich, Darmstadt, Germany) and sucrose 2% (A2211, Applichem GmbH, Darmstadt, Germany) in phosphate buffered saline (PBS) (18912-014, Gibco, Grand Island, NY, USA), and then washed once with PBS. Cells were thereafter permeabilized for 10 min with 0.5% Triton X-100 (X100, Merck, Saint Louis, MO, USA) in 100 Mm Tris-HCL pH 7.4 (A4263, Applichem GmbH, Darmstadt, Germany) and 50 Mm EDTA pH 8 (A4982, AppliChem GmbH, Darmstadt, Germany) in distilled water, washed again two times with PBS, and blocked overnight at 7°C with 0.5% bovine albumin (BSA) (A7906, Sigma-Aldrich, Darmstadt, Germany) and 0.2% gelatin from cold water fish (G7041, Sigma-Aldrich, Darmstadt, Germany) in PBS. Then, samples were stained with an antibody against histone H2AX (p S139) (NB100-384, Novus Biologicals, Abingdon, UK) in 0.5% BSA and 0.2% gelatin in PBS. After a 90 min-incubation at RT, cells were washed three times with PBS, and were thereafter treated for 90 min at RT with goat anti-rabbit IgG H&L cross-absorbed secondary antibody Rhodamine Red-X (R-6394, Thermo Fisher Scientific, Waltham, MA, USA) in 0.5% BSA and 0.2% gelatin in PBS. After immunostaining, cells were washed three times with PBS, and were stained with ProLong Gold Antifade Reagent with 4'6-diamidino-2-phenylindole (DAPI) (8961, Cell Signaling Technology Inc, Danvers, MA, USA).

Human Monocytes CRL-9855: H2AX phosphorylation detection was performed by using a modification of the aforementioned protocol. Cells were centrifuged on microscope slides using a Cytospin ROTANTA 460/460R centrifuge (Hettich, Tuttlingen, Germany). All cells were fixed for 15 min at RT with paraformaldehyde 3% (F8775, Sigma-Aldrich, Darmstadt, Germany) and sucrose 2% (A2211, Applichem GmbH, Darmstadt, Germany) in PBS (18912-014, Gibco, Grand Island, NY, USA), and then triple washed with PBS. Cells were thereafter permeabilized for 10 min with 0.5% Triton X-100 (X100, Merck, Saint Louis, MO, USA) in 100 Mm Tris-HCL pH 7.4 (A4263, Applichem GmbH, Darmstadt, Germany) and 50 Mm EDTA pH 8 (A4982, AppliChem GmbH, Darmstadt, Germany) in distilled water, washed again three times with PBS, and blocked overnight at 7°C with 0.5% BSA (A7906, Sigma-Aldrich, Darmstadt, Germany) and 0.2% gelatin from cold water fish (G7041, Sigma-Aldrich, Darmstadt, Germany) in PBS. Cells were incubated with an antibody against histone H2AX (p S139) (NB100-384, Novus Biologicals, Abingdon, UK) in 0.5% BSA and 0.2% gelatin in PBS for 90 min at RT and then washed three times with PBS. A 90 min incubation at RT with goat anti-rabbit IgG H&L cross-absorbed secondary antibody Rhodamine Red-X (R-6394, Thermo Fisher Scientific, Waltham, MA, USA) in 0.5% BSA and 0.2% gelatin in PBS followed. After three times washing with PBS, DNA was counterstained with ProLong Gold Antifade Reagent with 4'6-diamidino-2-phenylindole (DAPI) (8961, Cell Signaling Technology Inc, Danvers, MA, USA) and cells were shield with 22x22 mm coverslips, avoiding air bubbles trapping.

*Lymphocyte isolation*

Human peripheral blood samples were collected in lithium heparinized CPT vials (BD, Heidelberg, Germany) and lymphocytes were isolated according to the manufacturer`s manual. For the irradiation of 100 µl cell suspension in 48 well plates five million lymphocytes were diluted in 100 µl RPMI without

phenol red (#11835030, Gibco, Germany). After irradiation (only 0.5 Gy protons or 0.5 Gy protons followed by 100 or 400 J/m$^2$ UVB, respectively as well as vice versa: 100 or 400 J/m$^2$ UVB, respectively, followed by 0.5 Gy protons) 900 µl RPMI with phenol red (# 61870010, Gibco, Germany) was added. The suspension was divided into two wells, and the cells were kept for 2 h at 37°C, 5 % CO2, 95 % humidity for DNA repair.

*Dicentric chromosome assay*

The lymphocyte culturing was performed in the well plates without transfer into the usually used cell culture flasks to prevent cell loss of the just recently irradiated cells. After repair time replicate lymphocyte cultures were set up by adding 13 µL PHA (Phyothemagglutinin, #10576-015, Gibco, Germany) for lymphocyte stimulation before well plates were incubated for 48 h (37°C, 5 % CO2, 95 % humidity). After 24 h 500 µl fresh RPMI (including again 13 µl PHA) was added and Colcemide (final concentration 0.15 µg mL-1; #15212012, Gibco, Germany) was used to block lymphocytes at metaphase (MP) stage for the last 24 hours. Cultures were transferred into 1.5 ml reaction tubes (wells were washed with 500 µl 1x phosphate buffered saline, Gibco, Germany) and centrifuged (200 x g, 8 min). Hypotonic treatment was performed for 10 min at RT using 1 ml prewarmed (37°C) 0.075 M potassium chloride (#10575090, Gibco, Germany). After centrifugation (200 x g, 5 min), cells were fixed twice with freshly-prepared ice-cold fixative (3:1 methanol: acetic acid; 1 ml fixative was added and the solution was pipetted 3 times up and down). Cell suspensions were stored at -20°C at least overnight. After centrifugation (200 x g, 8 min) slides were prepared at RT and humidity of about 35 % by dispensing 40 µl of a suitable concentrated cell suspension onto clean wet slides and dried at least 2 h at RT. Dried slides were immersed in Giemsa stain (10% in GURR buffer, pH 6.8; #10582013, Gibco, Germany) for 3 min. After washing in three changes of A.d., the slides were air-dried for 2 h at RT. MP images were collected using the Metafer4 platform (MetaSystems, Altlussheim, Germany) coupled with a fully motorized Axio Imager Z.1 (Carl Zeiss), a PC controlled microscope for fully-automated MP finding, image acquisition and storing. For semi-automated DCA, scanned slides were run applying the DCScore software module from MetaSystems (Altlussheim, Germany). Chromosomes, indicated as “dicentric” by the DCScore were evaluated (confirmed or rejected) by a cytogeneticist. MP were not checked to be complete with 46 centromeres and observed false negative dicentrics were ignored.

**Reagents:**

*DNA Damage γH2AX Immunofluorescence assay*

- Formaldehyde solution (F8775, Sigma-Aldrich, Darmstadt, Germany)
- Sucrose (A2211, Applichem GmbH, Darmstadt, Germany)
- Phosphate buffered saline (PBS) (18912-014, Gibco, Grand Island, NY, USA)
- Triton X-100 (X100, Merck, Saint Louis, MO, USA)
- 100 Mm Tris-HCL pH 7.4 (A4263, Applichem GmbH, Darmstadt, Germany)
- 50 Mm EDTA pH 8 (A4982, AppliChem GmbH, Darmstadt, Germany)
- Bovine albumin BSA (A7906, Sigma-Aldrich, Darmstadt, Germany)
- Gelatin from cold water fish (G7041, Sigma-Aldrich, Darmstadt, Germany)
- Antibody against histone H2AX (p S139) (NB100-384, Novus Biologicals, Abingdon, UK)

- Goat anti-rabbit IgG H&L cross-absorbed secondary antibody Rhodamine Red-X (R-6394, Thermo Fisher Scientific, Waltham, MA, USA)
- ProLong Gold Antifade Reagent with 4’6-diamidino-2-phenylindole (DAPI) (8961, Cell Signaling Technology Inc, Danvers, MA, USA)

*Dicentric chromosome assay*

- BD Vacutainer® CPT™ (#BDAM362780, Becton Dickinson, Germany)
- RPMI-1640 medium with 200 mM L-glutamine (Gibco, Germany):
  - without phenol red (#11835030)
  - with phenol red (#61870010)
- DPBS (Dulbecco`s phosphate-buffered saline, #14190250, Gibco, Germany)
- PHA (Phyothemagglutinin, #10576-015, Gibco, Germany)
- Colcemide (final concentration 0.15 µg mL-1; #15212012, Gibco, Germany)
- 0.075 M potassium chloride (#10575090, Gibco, Germany)
- Methanol (#4627.6, Roth, Germany)
- Acetic acid (#3738.1, Roth, Germany)
- KaryoMAX Giemsa stain (Gibco, #10092013, Germany) in 10% in GURR buffer, pH 6.8(buffer tablets, #10582013, Gibco Germany)

References

1. Nikitaki Z, Pariset E, Sudar D, Costes SV, Georgakilas AG. In Situ Detection of Complex DNA Damage Using Microscopy: A Rough Road Ahead. Cancers (Basel). 2020 Nov 6;12(11):3288. doi: 10.3390/cancers12113288. PMID: 33172046; PMCID: PMC7694657.

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**Inventory of samples for gene expression**

**IVB**

| **July experiment (protons first, UVB second)** | **Cell line** | **Samples** | **Time post-irradiation** |
|---|---|---|---|
| 18.12.2023 | CRL 9855 3 experiments | Plate CTRL | 3 x 24h |
| | | 0.25 Gy | 3 x 24h |
| | | 0.25 Gy + 400 J/m$^2$ | 3 x 24h |
| | | 0.25 Gy + 800 J/m$^2$ | 3 x 24h |
| | | 400 J/m$^2$ | 3 x 24h |
| | | 800 J/m$^2$ | 3 x 24h |
| 20.07.2023 | CRL 9855 3 experiments | Plate CTRL | 3 x 24h |
| | | 0.25 Gy | 3 x 24h |
| | | 0.25 Gy + 100 J/m$^2$ | 3 x 24h |
| | | 0.25 Gy + 200 J/m$^2$ | 3 x 24h |
| | | 100 J/m$^2$ | 3 x 24h |
| | | 200 J/m$^2$ | 3 x 24h |
| | CRL 9855 2 experiments | Plate CTRL | 2 x 24h |
| | | 0.5 Gy | 2 x 24h |
| | | 0.5 Gy + 100 J/m$^2$ | 2 x 24h |
| | | 0.5 Gy + 200 J/m$^2$ | 2 x 24h |
| | | 100 J/m$^2$ | 2 x 24h |
| | | 200 J/m$^2$ | 2 x 24h |
| | CRL 9855 1 experiment | CTRL | 24h |
| | | 10 J/m$^2$ | 24h |
| | | 25 J/m$^2$ | 24h |
| | | 50 J/m$^2$ | 24h |
| | | 200 J/m$^2$ | 24h |
| | | 400 J/m$^2$ | 24h |
| 21.07.2023 | CRL 9855 1 experiment | CTRL | 24h |
| | | Plate CTRL | 24h |
| | | 0.5 Gy | 24h |
| | | 0.5 Gy + 100 J/m$^2$ | 24h |
| | | 0.5 Gy + 200 J/m$^2$ | 24h |
| | | 100 J/m$^2$ | 24h |
| | | 200 J/m$^2$ | 24h |
| **December experiment (protons first, UVB second)** | **Cell line** | **Samples** | **Time post-irradiation** |
| UVB curve 01.12.2023 | HaCaT | CTRL | 24h |
| | | Plate CTRL | 24h |
| | | 200 J/m$^2$ | 24h |
| | | 100 J/m$^2$ | 24h |
| | | 50 J/m$^2$ | 24h |

| | | | |
|---|---|---|---|
| | | 25 J/m$^2$ | 24h |
| | | 12.5 J/m$^2$ | 24h |
| | Hs-27 | CTRL | 24h |
| | | Plate CTRL | 24h |
| | | 400 J/m$^2$ | 24h |
| | | 200 J/m$^2$ | 24h |
| | | 100 J/m$^2$ | 24h |
| | | 50 J/m$^2$ | 24h |
| | | 25 J/m$^2$ | 24h |
| 04.12.2023 | HaCaT 3 experiments | CTRL | 2 x 24h + 3 x 48h |
| | | Plate CTRL | 2 x 24h + 3 x 48h |
| | | 0.5 Gy | 2 x 24h + 3 x 48h |
| | | 0.5 Gy + 25 J/m$^2$ | 2 x 24h + 3 x 48h |
| | | 0.5 Gy + 50 J/m$^2$ | 2 x 24h + 3 x 48h |
| | | 25 J/m$^2$ | 2 x 24h + 3 x 48h |
| | | 50 J/m$^2$ | 2 x 24h + 3 x 48h |
| | Hs-27 1 experiment | CTRL | 24h + 48h |
| | | Plate CTRL | 24h + 48h |
| | | 0.5 Gy | 24h + 48h |
| | | 0.5 Gy + 50 J/m$^2$ | 24h + 48h |
| | | 0.5 Gy + 100 J/m$^2$ | 24h + 48h |
| | | 50 J/m$^2$ | 24h + 48h |
| | | 100 J/m$^2$ | 24h + 48h |
| 06.12.2023 | HaCaT 1 experiment | CTRL | 24h + 48h |
| | | Plate CTRL | 24h + 48h |
| | | 0.5 Gy | 24h + 48h |
| | | 0.5 Gy + 25 J/m$^2$ | 24h + 48h |
| | | 0.5 Gy + 50 J/m$^2$ | 24h + 48h |
| | | 25 J/m$^2$ | 24h + 48h |
| | | 50 J/m$^2$ | 24h + 48h |
| | Hs-27 2 experiments | CTRL | 2 x 24h + 2 x 48h |
| | | Plate CTRL | 2 x 24h + 2 x 48h |
| | | 0.5 Gy | 2 x 24h + 2 x 48h |
| | | 0.5 Gy + 50 J/m$^2$ | 2 x 24h + 2 x 48h |
| | | 0.5 Gy + 100 J/m$^2$ | 2 x 24h + 2 x 48h |
| | | 50 J/m$^2$ | 2 x 24h + 2 x 48h |
| | | 100 J/m$^2$ | 2 x 24h + 2 x 48h |
| 07.12.2023 | HaCaT Proton curve | CTRL | 2 x 24h |
| | | Plate CTRL | 4 x 24h |
| | | 0.25 Gy | 3 x 24h |
| | | 1 Gy | 3 x 24h |
| | CRL9855 1 experiment | CTRL | 24h |
| | | Plate CTRL | 24h |
| | | 0.5 Gy | 24h |
| | | 0.5 Gy + 100 J/m$^2$ | 24h |

| | | | |
|---|---|---|---|
| | | 100 J/m$^2$ | 24h |
| 07.12.2023 | CRL9855<br>2<br>experiments | Plate CTRL | 2 x 24h |
| | | 0.5 Gy | 2 x 24h |
| | | 0.5 Gy + 50 J/m$^2$ | 2 x 24h |
| | | 50 J/m$^2$ | 2 x 24h |
| **April experiment (UVB first and protons second *versus* protons first and UVB second)** | **Cell line** | **Samples** | **Time post-irradiation** |
| 22.04.2024 | Hs-27<br>1 experiment<br>(protons first and UVB second) | CTRL | 24h + 48h |
| | | Plate CTRL | 24h + 48h |
| | | 0.5 Gy | 24h + 48h |
| | | 0.5 Gy + 50 J/m$^2$ | 24h + 48h |
| | | 0.5 Gy + 100 J/m$^2$ | 24h + 48h |
| | | 50 J/m$^2$ | 24h + 48h |
| | | 100 J/m$^2$ | 24h + 48h |
| | Hs-27<br>3<br>experiments<br>(UVB first and protons second) | CTRL | 3 x 24h + 3 x 48h |
| | | Plate CTRL | 3 x 24h + 3 x 48h |
| | | 0.5 Gy | 3 x 24h + 3 x 48h |
| | | 50 J/m$^2$ + 0.5 Gy | 3 x 24h + 3 x 48h |
| | | 100 J/m$^2$ + 0.5 Gy | 3 x 24h + 3 x 48h |
| | | 50 J/m$^2$ | 3 x 24h + 3 x 48h |
| | | 100 J/m$^2$ | 3 x 24h + 3 x 48h |
| 24.04.2024 | HaCaT<br>1 experiment<br>(protons first and UVB second) | CTRL | 24h + 48h |
| | | Plate CTRL | 24h + 48h |
| | | 0.5 Gy | 24h + 48h |
| | | 0.5 Gy + 50 J/m$^2$ | 24h + 48h |
| | | 50 J/m$^2$ | 24h + 48h |
| | HaCaT<br>3<br>experiments<br>(UVB first and protons second) | CTRL | 3 x 24h + 3 x 48h |
| | | Plate CTRL | 3 x 24h + 3 x 48h |
| | | 0.5 Gy | 3 x 24h + 3 x 48h |
| | | 50 J/m$^2$ + 0.5 Gy | 3 x 24h + 3 x 48h |
| | | 50 J/m$^2$ | 3 x 24h + 3 x 48h |
| 25.04.2024 | Hs-27<br>2<br>experiments<br>(UVB first and protons second) | CTRL | 2 x 24h + 2 x 48h |
| | | Plate CTRL | 2 x 24h + 2 x 48h |
| | | 0.1 Gy | 2 x 24h + 2 x 48h |
| | | 0.1 Gy + 50 J/m$^2$ | 2 x 24h + 2 x 48h |
| | | 50 J/m$^2$ | 2 x 24h + 2 x 48h |
| | HaCaT<br>2<br>experiments | CTRL | 2 x 24h + 2 x 48h |
| | | Plate CTRL | 2 x 24h + 2 x 48h |
| | | 0.1 Gy | 2 x 24h + 2 x 48h |
| | | 0.1 Gy + 50 J/m$^2$ | 2 x 24h + 2 x 48h |

| | | | |
|---|---|---|---|
| | (UVB first and protons second) | 50 J/$m^2$ | 2 x 24h + 2 x 48h |