Supplementary File 1

**Supplementary information:**

This file contains different lab protocols and procedures that were used in the study

**Procedure 1: Cultivation of adherent human cells (HEK293 cells)**

Medium used when culturing adherent cells in this study was the most commonly used:

DMEM (high glucose, **without** glutamine, e.g., Sigma/Merck D6546) or DMEM (high glucose, **without** glutamine and **without** sodium pyruvate, e.g., Sigma/Merck D5671)

The medium was supplemented with:

* 10 % (v/v) fetal calf serum, heat-treated for 30 min at 56 °C
* Penicillin/Streptomycin (Final concentrations: Penicillin: 100 U/ml, Streptomycin: 100 µg/ml)
* 2 mM L-glutamine (final concentration)
* if needed (see above): 1 mM sodium pyruvate (final concentration)

Additionally, supplemented medium for cell lines stably transfected with plasmids encoding geneticin, puromycin etc. resistance with the corresponding antibiotic.

**For stably transfected 293 cells:**

* G418-resistant stably transfected 293 cell lines are by default maintained in medium supplemented with 550 µg/ml G418, which is the concentration that is used for selecting them when they are generated.
* Puromycin-resistant stably transfected **293 cells** are by default maintained in medium supplemented with 2 µg/ml puromycin, which is the concentration that is used for selecting them when they are generated.

**Cultivation procedure:**

The procedure start with 9 ml complete medium (at least prewarmed to room temperature) is pipetted into a sterile 15 ml tube. Quickly a vial of cells are thawed. Cryotube with 70 % (v/v) EtOH (watch out, the label may disappear) are sprayed and the cells gets poured into the medium. Further on the cells gets spinned down (300 x g, 5 min). Supernatant is then removed and the cells get resuspended in complete medium. After this the cells are transferred into a 10 cm cell culture dish. The medium gets exchanged between 8 – 16 h after seeding (i.e., when cells are attached).

**Procedure 2: Maintenance/ subculturing cells**

Maintenance/ subculturing of cells is important in research. The cells for example should be split every 7-10 days at 370C. During this every surfaces of the laminar flow hood has to be wiped down with 70% EtOH. Also all items that is introduced into the hood must be disinfected 70% EtOH e.g. media, containers and pipettors. The work area must be loaded withappropriate pipettes, bottles, 10 cmculture dish etc. The culture medium for the cells must be optically clear and free of evidence of microbial or fungal contamination. The cell sheet must be examined with an inverted light microscope. Culture should be 70-80% confluent.

During the subculturing the medium is aseptically aspirated out. Then cells are washed with

sterile 1x PBS and added 1.5 ml 0.05% trypsin/EDTA solution. This is spread evenly and incubated at 37 0C. **NB:** Cells will appear rounded when examined under inverted light microscope. The cells are then examined under microscope and trypsin gets inactivated by adding 8.5 ml FBS supplemented medium. If necessary the culture dish is carefully knocked until the cells are loosened. The split ratio of the cells is at 1:10

**CELL QUANTIFICATION USING A NEUBAUER CHAMBER, 0.100 mm DEPTH, 0.0025 mm2/HAEMOCYTOMETER**

Cell quantification is done precede transfection, cryopreservation, and subculture routines. Cells are trypsinated and diluted for sufficient segregation. The haemocytometer and glass cover is cleaned with 70% EtOH. The coverslip is then moisten with water or exhaled breath. The coverslip gets slided over the chamber back and forth using slight pressure until Newton’s refraction rings appear (rainbow-like rings). Both sides of the chamber are then filled with cell suspension, approximately 10 μl on the loading groove. It is important to allow sample to diffuse by capillarity.

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**Quantification under the microscope**

Under an inverted phase contrast microscope using 10x objective; the nine main squares of counting chamber delimited by three lines gets identified (Fig. 1).

**NB:** Cells that touch the upper and left border are counted (black dots, Fig.2) whilst cells that touch the right and lower border are not counted (red dots).

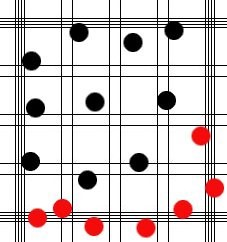
This procedure is performed in four main squares. If the number is too high a dilution is considered. The count should proceed from the top left-hand corner and follow the direction in Fig. 3.

**Calculating number of cells:**

To calculate the number of cells this is done by multiplying the dilution factor by the total number of cells, divided by the # of corner squares counted, and multiplied by 104 to obtain cell concentration (cells/ml).

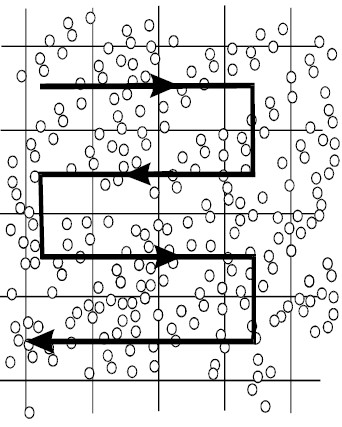
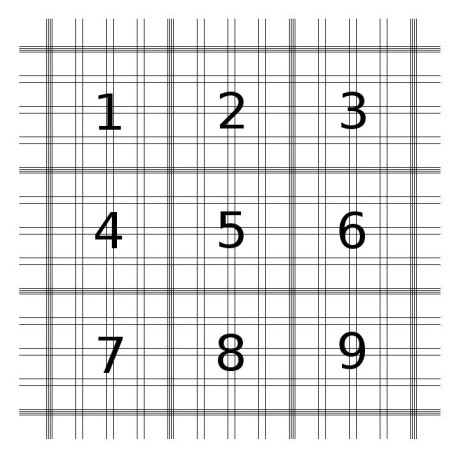
cell concentration equation

Clean haemocytometer and glass cover slip with 70% EtOH.

**Fig. 1****Fig. 2**

**Fig.**

**3**



**Procedure 3: Transfection of cells with peroxisomal and mitochondrial PARP1cd plasmids using XtremeGene9 reagent**

This procedure spans over three days, where the first day involves seeding cells into 6 well plates. The second day involves transfecting NUDT12 cells with the plasmid DNA pEX-EGFP-PARPcd shown in the Figure below. The third day involves determining the protein concentration of the samples

**Day 1:**

1. 800,000 HEK293 cells is seeded in 6 well plate the day before transfection. The cells are grown in a total volume of 2-2.5 ml medium.

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1. Below is the experimental setup for the two 6-well plates:

# PLATE 1

|  |  |  |
| --- | --- | --- |
| 293-Parental (i.e., wildtype control) | 293-Parental + PexPARP |  |
| 293+NUDT12 | 293+NUDT12 + PexPARP |  |
| **PLATE 2** |  |  |
| 293-Parental | 293-Parental + mitoPARP |  |
| 293-SLC25A51KO | 293-SLC25A51KO + mitoPARP |  |

**Day 2:**

The plasmid DNA solution gets prepared using sterile water at a concentration of 0.5 μg/μl. The medium is then removed and carefully added 2 ml fresh medium to the cells. The X-tremeGENE 9 DNA Transfection Reagent vial is briefly vortexed and in a sterile tube, 3 µl X-tremeGENE 9 DNA Transfection Reagent is diluted in 100 µl Opti-MEM and mixed gently by vortexing. Further a total volume of diluted X-tremeGENE 9/Opti-MEM that is suffcient for the number of transfections planned is prepared. 1 μg of plasmid DNA to 100 μl of diluted X-tremeGENE 9 DNA Transfection Reagent is added. This is vortexed gently (i.e., avoid any droplets getting stuck in the lid or at the inner wall of the tube). The transfection reagent:DNA complex gets incubated for 20 minutes at room temperature. After this the transfection complex is added to the cells in a dropwise manner. The transfection complexes is then distributed by gently shaking the 6 well plate.

**Day 3:**

Cells are checked under a fluorescent microscope to confirm successful transfection. Protein lysate is made by adding 150 µL of lysis buffer supplemented with 1 mM 3aminobenzamide, an inhibitor of endogenous poly ADP ribose polymerase (PARP). This lysate is passed through a 23-gauge needle to reduce viscosity. Protein concentration is determined byusing the BCA assay. Each sample is diluted to 50 µg protein for SDS-PAGE.

**Procedure 4: MitoTracker staining**

MitoTracker is added one day day post transfection / cell seeding. This is done by replacing the culture medium with prewarmed medium supplemented with 0.2 µM MitoTracker

The stock solution is prepared as a 200 µM solution usually diluted 1:1000 with the medium. The cells are incubated at 37 °C in the cell incubator for 30 min. Carefully cells are washed with prewarmed medium. Cells are then fixed with 4% paraformaldehyde (or 4% formaldehyde) in PBS.

After adding MitoTracker try to protect the cells as good as possible from light.

**Procedure 5: Immunocytochemistry of transiently transfected cells**

This procedure spans over three days, where the first day involves seeding cells into a 24 well plate. The second day involves transfecting NUDT12 cells and the third day involves immunodetection with immunocytochemistry.

**Day 1:**

Cover slips are placed into the cavity of a 24 well plate. Cells are seeded (e.g., 75,000 HeLa S3 or 150,000 293 cells) in the cavity. Medium are added and the final volume is 1 ml.

**Day 2**

Cells are transfected with the transfection reagent and incubated for at least 24 h.

**Day 3: Immunocytochemistry**

All steps are performed in the cavity of a 24-well plate

Wash the cells with 1 ml PBS

30 min 4 °C Fix the cells with 500 µl 4 % (v/v) ice-cold formaldehyde or paraformaldehyde solution prepared in 1x PBS

1 x Wash with 1 ml PBS

15 min RT Permeabilize cells with 500 µl 0.5 % (v/v) Triton X-100 (v/v) in PBS

1 x Wash with 1 ml PBS

1 h RT Block with 1 ml complete medium

[i.e., supplemented with 10 % (v/v) FCS]

**Immunodetection (part 1):**

at least 2 h at RT or Dilute primary antibody in complete medium overnight at 4 °C-10 °C [i.e., supplemented with 10 % (v/v) FCS]

Add at least 250 µl diluted antibody solution to the cells

1 x Wash with 1 ml PBS (3 min)

1 x Wash with 1 ml 0.1 % (v/v) Triton X-100 (v/v) in PBS (3 min)

1 x Wash with 1 ml PBS (3 min)

**Immunodetection (part 2):**

1 h RT Dilute secondary antibody in complete medium

[i.e., supplemented with 10 % (v/v) FCS]

Add at least 250 µl diluted antibody solution to the cells

[optional, but recommended: stain nuclei with DAPI diluted 1:20,000 in PBS (500 µl), 5-10 min RT]

1 x Wash with 1 ml PBS (3 min)

1 x Wash with 1 ml 0.1 % (v/v) Triton X-100 (v/v) in PBS (3 min) 1x Wash with 1 ml PBS (3 min)

Mount the cover slips onto glass slides using a commercial mounting solution or a homemade mounting solution (see below for protocol)

**DAPI:**

* stock solution: 5 mg/ml in DMF
* dilute to a final concentration of 0.5 µg/ml (1:20,000) in PBS

**Mounting solution:**

* add 2.4 g Mowiol (G488) to 4.8 ml 100 % glycerol
* add 6 ml H2O → stir at RT for several hours
* add 12 ml 0.2 M Tris-HCl pH 8.5 → 50° C → stir until Mowiol is dissolved
* add 0.45 g DABCO [final conc. = 2.5 % (w/v)] → 4° C → stir until DABCO is dissolved • store aliquots at -20° C

**Antibodies used in the study**

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Automatisk generert beskrivelseThe figure show the different antibody used in the immunostaining. The ones used in the study is highlighted in yellow. The complete Excel file can also be found in link to the results.

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