Electronic Supplementary Information

Cellular Toxicity

The extent of cellular toxicity caused by transfection is influenced by the reagent and the nature of the cells. In particular, for cell types, dramatic toxicity can be directly visualized by using microscopy. Cytotoxicity of pcDNA6/TR is reported in Figure 1 of the main text. We also evaluated cytotoxicity by light microscopy of transfected cells, both uninduced and induced. Transient transfection with pcDNA5/TO-MRS2 with PEI as the transfection reagent did not alter the proliferation and morphological features of Clone-2 cells, both induced and uninduced. HEK-293 cells are reported as an additional control.

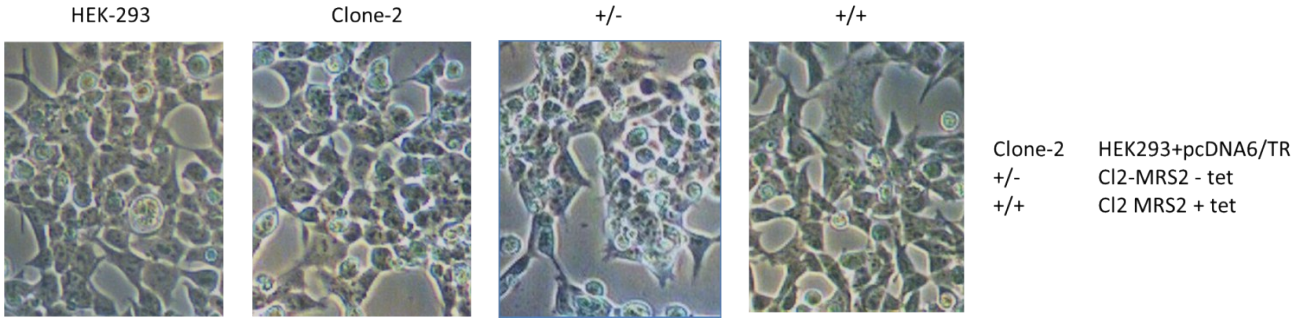

Fig. 10 ESI Cell morphology of HEK293, Clone-2, Clone-2 transfected with pcDNA5/TO -MRS2, un-induced, and induced. Treatment does not induce any cytotoxicity.

Real time-qPCR

Cells were seeded at a density of 2.5 x 10^6 cells per T75 flask and grown overnight. PEI-mediated transfection with either pcDNA5/TO-MRS2, pcDNA5/TO-SLC41A3, or the empty vector control was carried out as described. Twenty-four hours after transfection, protein expression was induced by the addition of tetracycline, whereas uninduced control cells were left untreated. After a further 24 hours, cells were collected, washed two times with ice-cold PBS, and immediately frozen in liquid nitrogen. Total RNA was isolated with the Nucleospin RNA II Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The RNA concentration was quantified by NanoDrop (PEQLAB Biotechnologie GmbH, Erlangen, Germany), and RNA integrity (RIN) was determined with a 2100 Bioanalyzer (Agilent Technologies, Böblingen, Germany). All samples had a RIN value of 9 or higher. cDNA was synthesized with the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories GmbH, Munich, Germany) by reverse-transcribing 1000 ng total RNA in a volume of 20 µl (in an Eppendorf Mastercycler nexus gradient; Hamburg, Germany). The cDNAs were then diluted 1:5. The primer pairs used for RT-qPCR were first tested in conventional PCRs for their specificity, and the PCR products were analyzed by bidirectional sequencing.

Used primers:

MRS2 *(MRS2*Fwd: 5'-CCACCGAAACGTGATGATGAG-3'; *MRS2*Rev: CATTCCAAAAGCAACTCCC-3'; amplicon size 94 bp)

SLC41A3 *(SLC41A*3Fwd: 5'-CTTCTGGACTATTTCCAGCAC-3'*; SLC41A3*Rev: 5'-TCATCTCCAGGTTCCCCTTC-3'; amplicon size 100 bp)

The reference genes *B2M* (NM_004048, Beta-2-microglobulin) and *YWHAZ* (NM_001135699.1, Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, zeta polypeptide) and *Tuba1b* (NM_006082.2, Tubulin, alpha 1b) were used in the qPCRs.

B2M (*B2M*Fwd: 5'-TGCTGTCTCCATGTTTGATGTATCT-3'; *B2M*Rev: 5'-CTCTGCTCCCCACCTCTAAGT-3'; amplicon size 86 bp)

YWHAZ (*YWHAZ*Fwd: 5'-ACTTTTGGTACATTGTGGCTTCAA-3'; *YWHAZ*Rev: 5'-CCGCCAGGACAAACCAGTAT-3'; amplicon size: 94)

Tuba1b (*Tuba1b*Fwd: 5'-GCCCTACAACTCCATCCTCA-3'; *Tuba1b*Rev: 5'-GTCAACATTCAGGGCTCCAT-3'; amplicon size: 205)

For qPCR experiments, a 40-cycle three-step PCR protocol (30 s at 95 °C, 30 s at 58 °C, and 30 s 72 °C) was performed on a thermocycler (ViiA7, Applied Biosystems/Life Technologies) with SYBR green detection and three replicates per reaction. The final volume was 10 µl containing 5 µl cDNA, primers (0.5 µl of 20 pmol/µl each), and iQ SYBR Green Supermix (Bio-Rad Laboratories GmbH, München, Germany). Thresholds were automatically calculated by the cycler software. For data analysis, the software qbasePLUS (Biogazelle NV, Zwijnaarde, Belgium) was used to perform inter-run calibration and to test for the expression stability of the reference genes. Normalization was performed with *B2M* and *YWHAZ*, since *Tub1b* did not exhibit sufficient stability. After the normalization of Cq values with the respective reference genes, results were exported as calibrated normalized relative quantity (CNRQ) values. Relative expression values were used for statistical analysis.

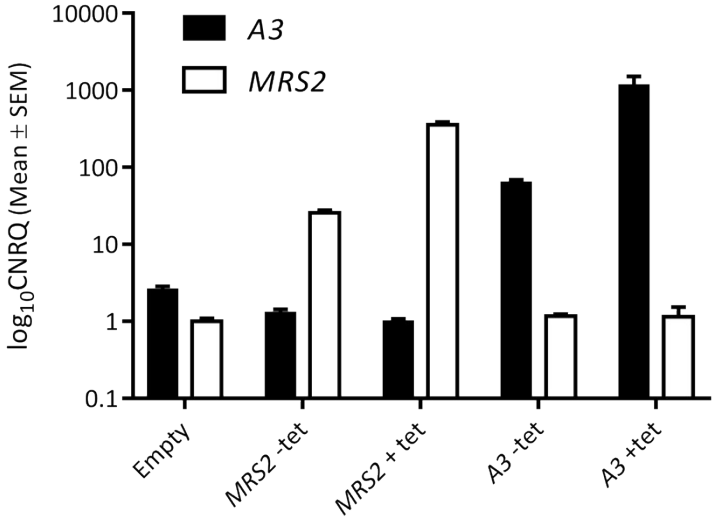

Fig. 11 ESI qPCR analysis of *SLC41A3* (*A3*) and *MRS2* expression. Averaged normalized logarithmic relative expression values of *A3* and *MRS2* in arbitrary units (CNRQ ± SEM) determined by qPCR analysis of Clone-2 cells, induced (+tet) and uninduced (-tet). Clone-2 cells transfected with empty vector were used as controls.

DCHQ5 Chemical Structure

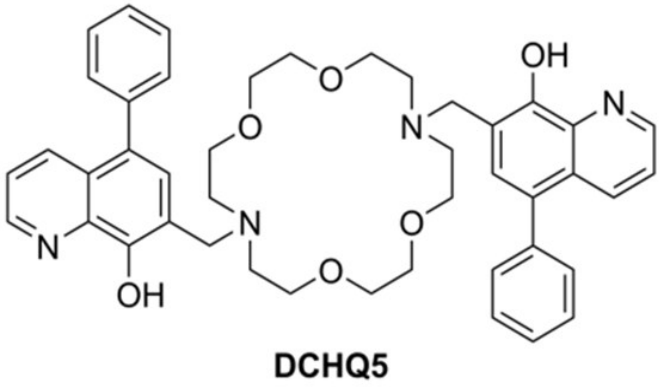

Fig. 12 ESI DCHQ5 structure. Chemical structure of the fluorescent chemosensor Diaza-crown-8 hydroxiquinoline derivative.

Single Cell Analysis

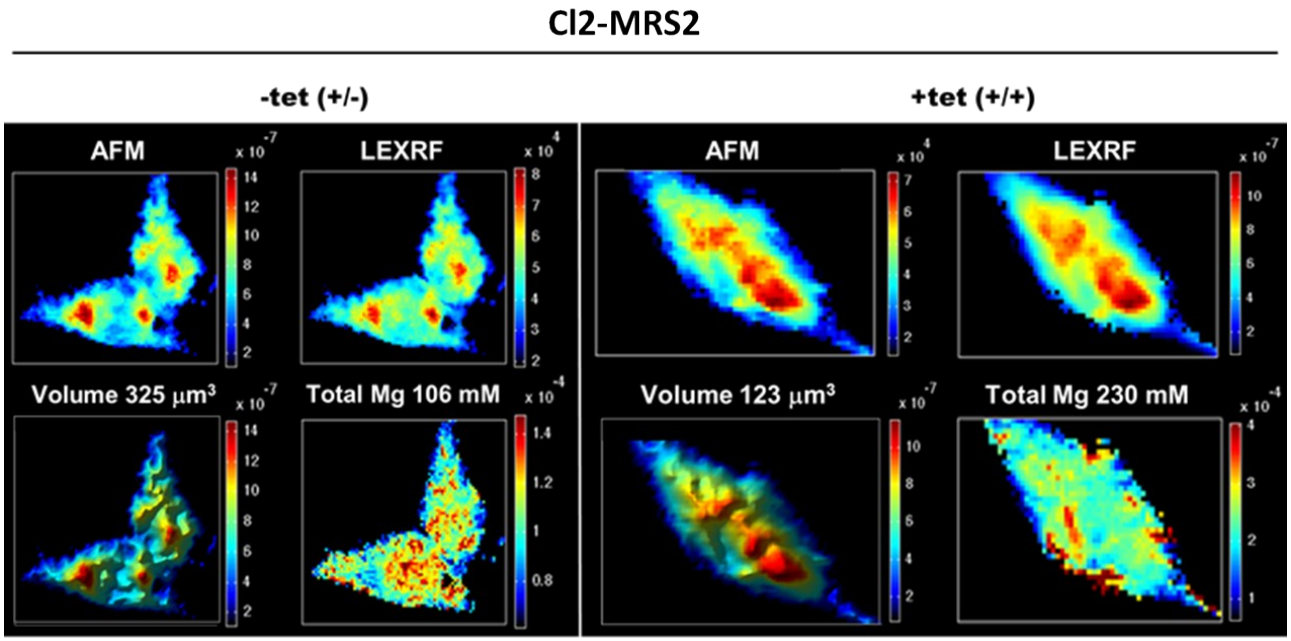

Fig. 13 ESI. Single cell analyses of two chosen uninduced and induced Cl2-MRS2 cells are reported for completeness. The maps confirm the higher distribution of Mg in the nuclear region with spots in the peripheral area in both induced and un-induced cells. Single cell analysis performed on chemically fixed Cl2-MRS2 cells, both uninduced (-tet +/-) and induced (+tet +/+) with tetracycline at 1μg/mL. The upper panels show the AFM (right) and LEXRF (left) maps. The bottom panel depicts a 3D rendering of the AFM (left) and the Mg intracellular concentration (right) maps, mathematically estimated from the combination of AFM and LEXRF analyses and highlighting the cell thickness and the overall distribution of the intracellular Mg, respectively. The total Mg concentration represents the concentration value of the entire single cell. Single cell analyses were carried out on six uninduced (-tet +/-) and seven induced (+tet +/+) Cl2-MRS2 cells. Scale bar is 2 μm.

Cell volume assessment

To determine the hydrated cell volume for cell population analysis, Clone-2-MRS2 cells were trypsinized and resuspended in PBS. Cells volumes were calculated by counting Cl2-MRS2 cells on a double-threshold Z1 Coulter Counter (Beckman Coulter, CA, USA), and the thresholds were set to cover the interval from 65 to 3,600 fL, each step corresponding to an increase of 2 µm in cell diameter. The mean cellular volume was estimated from the Gaussian distribution of the data. The analysis were carried out in triplicate. The cellular volume is given in terms of equivalent spherical diameter.

The volume of dehydrated Clone-2-MRS2 cells (for single cell analyses) was calculated on chemically fixed cells by using the AFM imaging technique. Measurements were carried out in air in tapping mode by using an XE-100 instrument (Park Instruments) operating in soft-contact mode (force setpoint= 0.1-1nN) by silicon cantilevers (CSC38 Mikromasch, spring constant= 0.03 N/m). The typical square scan size used was in the order of 40 µm × 40 µm, and the matrix resolution in pixels were 256x256 or 512 × 512.

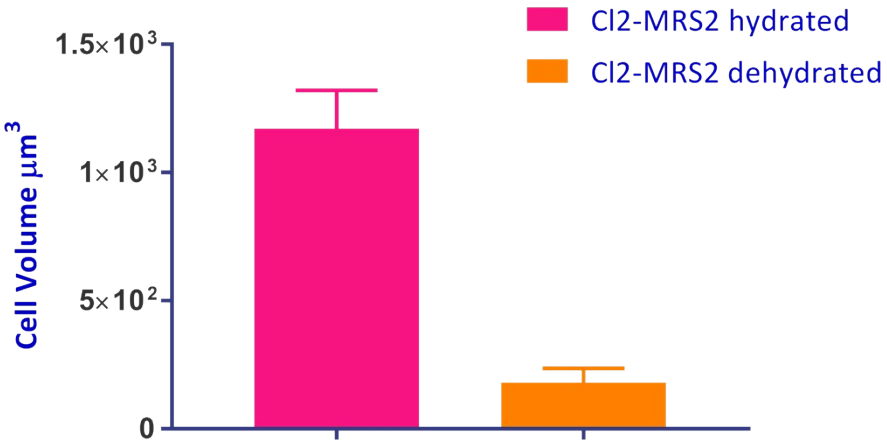

Fig. 14 ESI Mean cellular volume (μm^3) of Clone2-MRS2 cells, hydrated (pink) and dehydrated (orange).