

## SHORT COMMUNICATION

### **SULT4A1 Protects Against Oxidative-Stress Induced Mitochondrial Dysfunction in Neuronal Cells**

Mohammed I. Hossain<sup>1</sup>, Joshua M. Marcus<sup>1</sup>, Jun Hee Lee<sup>1</sup>, Patrick L. Garcia<sup>1</sup>, Jean-Philippe Gagné<sup>3</sup>, Guy G. Poirier<sup>3</sup>, Charles N. Falany<sup>1</sup>, Shaida A. Andrabi<sup>1,2,4</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA

<sup>2</sup>Department of Neurology, University of Alabama at Birmingham, Birmingham, AL, 35294, USA

<sup>3</sup>Centre de recherche du CHU de Québec, Faculté de Médecine, Université Laval, Québec, Canada

**Running title: SULT4A1 Preserves Mitochondrial Function**

**Address correspondence to:** Shaida A. Andrabi, PhD

Dept. of Pharmacology and Toxicology, 1670 University Blvd., Volker Hall Room 242,  
University of Alabama at Birmingham, Birmingham, AL 35294, USA. Tel (205) 975-3639;  
FAX (205) 934-8240; E-mail: [sandrabi@uab.edu](mailto:sandrabi@uab.edu)

**Number of text pages: 16**

**Number of figures: 2**

**Number of references: 20**

**Number of words in Abstract: 176/250**

**Number of words in Significance statement: 83/120**

**Number of words in Introduction: 474**

**Number of words in Results and Discussion: 978/1500**

**Abbreviations:** ATP, adenosine tri-phosphate; Ab, antibody; Cas, content-addressable storage; CCCP, carbonyl cyanide m-chlorophenyl hydrazine; CRISPR, clustered regulatory interspaced short palindromic repeats; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetra acetic acid; ETC, electron transport chain; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HCl, hydrochloric acid, HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; hSULT4A1, human SULT4A1; KCl, potassium chloride; NaCl, sodium chloride; MgCl<sub>2</sub>, magnesium chloride, LSM, laser scanning microscope; MOI, multiplicity of infection; OCR, oxygen consumption rate; PAP, 3'-phosphoadenosine-5'-phosphate; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SULT, cytosolic sulfotransferase; SULT4A1, sulfotransferase 4A1; TMRE, tetramethylrhodamine ethyl ester; TOM20, translocase of the outer membrane of mitochondria

20

**Abstract**

Sulfotransferase 4A1 (SULT4A1), a member of cytosolic sulfotransferases (SULT) is exclusively expressed in neurons with no known function. Severe phenotype and early postnatal death in SULT4A1 knock out mice revealed that SULT4A1 is an essential neuronal protein. Localization of SULT4A1 in different cytosolic compartments, including mitochondria, suggests multiple roles for this protein. We observed that knockdown of SULT4A1 results in the accumulation of reactive oxygen species in primary cortical neurons, suggesting a potential role of SULT4A1 in regulating redox homeostasis. Expression of SULT4A1 in the human neuroblastoma SH-SY5Y cells revealed a defused but non-uniform staining pattern in the cytoplasm, with increased density around mitochondria. Subcellular fractionation of SULT4A1 expressing SH-SY5Y cells confirms the presence of SULT4A1 in mitochondrial fractions. SULT4A1 expressing cells display significant protection against H<sub>2</sub>O<sub>2</sub>-mediated defects in mitochondrial function and loss of mitochondrial membrane potential. Expression of SULT4A1 in SH-SY5Y cells also protects against H<sub>2</sub>O<sub>2</sub>-induced cell death. These data indicate that SULT4A1 protects mitochondria against oxidative damage, and may serve as a potential pharmacological target in neural diseases involving mitochondrial dysfunction and oxidative stress.

### **Significance statement**

Studies on SULT4A1 knock out mice suggest that SULT4A1 plays a vital role in neuronal function and survival via yet undefined mechanisms. Our data demonstrate that depletion of SULT4A1 induces oxidative stress in neurons and expression of SULT4A1 in SH-SY5Y cells protects against oxidative-stress-induced mitochondrial dysfunction and cell death. These results suggest that SULT4A1 may have a crucial protective function against mitochondrial dysfunction and oxidative stress, and may serve a potential therapeutic target in different neurological diseases involving mitochondrial dysfunction and oxidative stress.

## Introduction

Sulfotransferase (SULT) 4A1 is a member of the cytosolic SULT gene family based on its structural homology (Allali-Hassani et al., 2007). Cytosolic SULTs catalyze the transfer of a sulfonate group from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to their substrates (Suiko et al., 2017). SULT4A1 is highly conserved and selectively expressed in neurons in the brain of vertebrates (Garcia et al., 2018). SULT4A1 has a full length and a splice variant transcript that is expressed in different tissues and cell types. The full-length SULT4A1 variant found only in the neurons, encodes the complete protein sequence whereas the splice variant transcript, found in other tissues and cells encodes an unstable protein that is undetectable. The instability of the splice variant protein is attributed to the insertion of a premature stop codon due to the failure of correctly excising an intron between exons 6 and 7 (Falany et al., 2000; Sidharthan et al., 2014). No known enzymatic activity of SULT4A1 has been reported since its initial cloning and characterization by Falany et al. in 2000 (Falany et al., 2000). SULT4A1 lacks PAPS or binding properties due to the absence of a part of loop 3 that covers the substrate-binding pocket indicating that SULT4A1 may not be a catalytically active SULT (Garcia et al., 2018). Changes in behavioral activity in SULT4A1-deficient zebrafish (Crittenden et al., 2015), and a profound neuronal phenotype and postnatal death observed in CRISPR / Cas9 generated SULT4A1 knockout (KO) mice revealed that SULT4A1 is a vital neuronal protein (Garcia et al., 2018). Unlike other cytosolic SULTs, SULT4A1 localizes with different subcellular organelles including mitochondria (Garcia et al., 2018), suggesting that SULT4A1 may have multiple novel functions in the brain.

Mitochondrial vigor is crucial for neural survival and functioning as the brain on average uses nearly 20% of the energy consumed by the body even though brain mass accounts for only 2% of the total body weight (Raichle and Gusnard, 2002). Most of the ATP in neurons is used to maintain resting potential, propagation of nerve impulse transmission and neurotransmitter

release (Raichle and Gusnard, 2002). Additionally, axonal transport and mitochondrial dynamics in neurons are bio-energetically demanding processes (Raichle and Gusnard, 2002). Therefore, neuronal mitochondria may require additional factors for their efficiency and protection to support the demanding bioenergetic needs. SULT4A1 shows novel mitochondrial localization (Garcia et al., 2018), and there is an increase in oxidative stress following knockdown of SULT4A1 in mouse cortical neurons. Therefore, regulation of mitochondrial function and redox homeostasis may comprise important functions of SULT4A1 in neurons. We used SH-SY5Y neuroblastoma cells to evaluate the mitochondrial roles of SULT4A1 in normal and H<sub>2</sub>O<sub>2</sub>-induced oxidative stress conditions. Our results indicate that SULT4A1 expression in SH-SY5Y cells increases maximal mitochondrial respiration and protects against H<sub>2</sub>O<sub>2</sub>-induced mitochondrial dysfunction. These studies suggest that regulation of mitochondrial function in stress conditions and redox homeostasis are important SULT4A1 functions in neurons.

## Materials and Methods

**Cell Culture and Lentiviral Transduction.** SH-SY5Y cells were grown in DMEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 5mM glucose and 10% FBS. Human SULT4A1 was expressed in SH-SY5Y cells using a lentiviral transduction as described previously (Hossain et al., 2013). Briefly, human SULT4A1 was cloned into pLVX-Puro lentiviral vector along with psPAX2 lentiviral packaging vector, pMD2.G lentiviral envelope vector co-transfected into HEK293FT cells using 25 kDa linear polyethylenimine (Polysciences, Warrington, PA) transfection reagent. Virus was collected from the media and directly added to SH-SY5Y cells. Transduced cells were selected using puromycin (Sigma, St. Louis, MO). Control virus was packaged in the same way using empty pLVX-Puro vectors. H<sub>2</sub>O<sub>2</sub> (500  $\mu$ M for 2 hrs.) was used to induce oxidative stress in these cells. Seahorse assay, TMRE imaging was performed immediately after H<sub>2</sub>O<sub>2</sub> treatment, and cell death was assessed after 24 hours.

**Primary Cortical Neuronal Culture and shRNA knockdown.** Primary cortical neurons were cultured from embryonic day 15 mouse embryos as previously described (Andrabi et al., 2014; Garcia et al., 2018). Cells were plated at a density of  $5 \times 10^5$  cells/ml on poly-L-Ornithine (Sigma, P3655) coated cell culture plates in neurobasal medium, supplemented with, 1 mM GlutaMax, 1 mM Sodium Pyruvate, B-27 and 10 mM glucose (Thermo Scientific, USA). 5-fluoro-2-deoxyuridine (40  $\mu$ M) (Sigma, F0503) was used to inhibit glial growth at DIV 2. Lentiviruses containing control shRNA (pLKO.1 GFP shRNA, Addgene, USA) or SULT4A1 shRNA (TRCN0000103353, Sigma, USA) were transduced (MOI of 5) on DIV 6. Experiments were performed on DIV 10.

**ROS Measurement.** Reactive oxygen species (ROS) were measured using CellRox Deep Red (Thermo Scientific, USA) imaging. Neurons were incubated with 5  $\mu$ M CellRox for 30 min at

37°C. Live-cell images were captured using an LSM 710 Confocal Microscope (Carl Zeiss, Germany). Fluorescence intensity was quantified using ImageJ software (NIH).

**Subcellular Fractionation and Western Blotting.** The SH-SY5Y cells were suspended in fractionation buffer containing 20 mM HEPES (pH 7.4), 10 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM EDTA, and 1 mM EGTA, 1 mM dithiothreitol and complete protease and phosphatase inhibitor (Thermo Fisher Scientific). The cell suspension was passed through a 30 G needle 10 times followed by centrifugation at 750× g for 5 minutes at 4°C. The supernatant containing cytoplasm, membrane and mitochondria was further centrifuged at 10,000 × g for 10 minutes at 4°C. The resultant pellet containing mitochondria was re-suspended in lysis buffer containing 25 mM Tris HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% Sodium Deoxycholate, 1 mM EDTA, 1 mM EGTA and complete phosphatase and protease inhibitor. Protein concentrations were determined by BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein were separated by SDS-PAGE gel electrophoresis and transferred onto a nitrocellulose membrane. A rabbit anti-SULT4A1 polyclonal antibody (Proteintech, USA) followed by a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Cell Signaling, Beverly, MA) was used to detect SULT4A1 via SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). GAPDH (Cell Signaling) and TOM20 antibodies (Proteintech, USA) were used for cytosolic and mitochondrial fractions, respectively (Dimauro et al., 2012; Wang et al., 2016).

**Immunocytochemistry.** SH-SY5Y cells expressing SULT4A1 were fixed in 4% paraformaldehyde for 15 minutes and permeablized with 0.2% Triton X-100 for 30 minutes. Following blocking, the cells were incubated in anti-SULT4A1 and anti-TOM20 antibodies (Proteintech, USA) overnight at 4°C. Alexa Fluor 488 donkey anti-mouse secondary antibody and Alexa Fluor 555 donkey anti-rabbit secondary antibody (Thermo Fisher Scientific) were used to detect the primary antibodies. Coverslips were mounted on glass slides using Immuno-



Mount mounting medium (Thermo Fisher Scientific). LSM 710 confocal microscope (Carl Zeiss, Germany) was used for imaging.

**Mitochondrial Function.** Mitochondrial function was measured as Oxygen Consumption Rate (OCR) in SH-SY5Y cells using an XF<sup>96</sup> Extracellular Flux Analyzer (Agilent Technologies, Santa Clara, CA). 70,000 cells were plated in each well of an XF<sup>96</sup> cell culture microplate (Agilent Technologies) and maintained overnight in a 5% CO<sub>2</sub> at 37°C in a humidified incubator. Cells were washed and placed in serum-free, pyruvate-free, glutamine-free DMEM (Agilent Technologies). Oligomycin, CCCP, and rotenone/antimycin A (Sigma Aldrich, USA) were injected into each well sequentially to determine basal respiration, maximal respiration, spare respiratory capacity ATP turnover and proton leak as described previously (Andrabi et al., 2014).

**Mitochondrial Membrane Potential ( $\Delta\Psi_m$ ).** Tetramethylrhodamine Ethyl Ester (TMRE) 20 nM (Thermo Fisher Scientific) was incubated with SH-SY5Y cells for 30 min at 37°C. TMRE live-cell imaging was acquired using an LSM 710 confocal microscope Carl Zeiss, Germany). Images were captured at 10 sec intervals for 1 min to get a baseline, 20  $\mu$ M CCCP was added to depolarize mitochondria and images were captured for an additional 2 minutes.  $\Delta\Psi_m$  was calculated as fold change in TMRE intensity before and after CCCP addition.

**Cell Death Assays.** Alamar blue reagent (Thermo Fisher Scientific) was used to assess cell viability. Alamar blue reagent [10% (v/v)] was incubated in the cultures for 3 h. 100  $\mu$ L of the medium was collected from each well and transferred to a 96-well microplate. Victor X5 microplate reader (Perkin Elmer, MA, and USA) was used to measure the fluorescence using excitation and emission wavelength of 540 and 595 nm respectively. Data was quantified and presented as percent control.

## Results and Discussion

SULT4A1 is an important neuronal-specific protein (Garcia et al., 2018). Loss of SULT4A1 in SULT4A1 knockout mice results in tremors, abnormal gait, rigidity, and death at 3-4 weeks of age (Falany et al., 2000). Despite the apparent importance of SULT4A1 in neurons, no SULT catalytic activity has been reported for this vital neuronal protein. It is likely that SULT4A1 is a catalytically inactive member of SULT family and functions by regulating some important neural functions by yet undefined mechanisms. The distribution of SULT4A1 is diffused in the cytosol of neurons with substantial mitochondrial and microsomal localization (Garcia et al., 2018). We used shRNA-mediated knockdown of SULT4A1 via lentiviral transduction in mouse cortical neurons (Fig 1A) and observed that ROS accumulate in these neurons (Fig 1B & C), suggesting that SULT4A1 may help in maintaining neuronal redox homeostasis. SULT4A1 localizes to the mitochondria, and these organelles are one of the principal sources of ROS in a cell (Nohl et al., 2005; Murphy, 2009). One possibility is that SULT4A1 protects mitochondria against oxidative damage and maintains redox homeostasis in the neural cells. To assess these function, we used lentiviral transduction to express SULT4A1 in neuroblastoma SH-SY5Y cells (Fig 1D). Subcellular fractionation and western blotting revealed that SULT4A1 localizes to the mitochondrial fraction in SH-SY5Y cells expressing SULT4A1 (Fig 1E). Likewise, immuno-fluorescence staining demonstrated that transduced SULT4A1 in SH-SY5Y cells displays diffuse cytosolic staining with a considerable co-localization with the mitochondrial outer membrane protein, TOM20 (Fig 1F). Orthogonal projections revealed that SULT4A1 co-localizes with TOM20 in XY, XZ, and YZ planes (Fig 1F). These data confirm our previous results in mouse brain (Garcia et al., 2018) and suggest that the spatiotemporal localization of SULT4A1 expressed in SH-SY5Y cells is comparable to its endogenous localization in neurons.

As the function of SULT4A1 in neurons has been an enigma in the field, it does appear to have an important role in regulating neuronal functions, including bioenergetics and redox balance. To determine whether SULT4A1 affects mitochondrial function and possibly protects against oxidative stress, mitochondrial function in SH-SY5Y cells that express SULT4A1 was monitored in presence or absence of H<sub>2</sub>O<sub>2</sub> using an XF Seahorse flux Analyzer. H<sub>2</sub>O<sub>2</sub> is a potent oxidative stress agent and induces ROS generation in the cells (Wazawa et al., 1992). We monitored mitochondrial function as oxygen consumption rate (OCR) using a comprehensive Seahorse Flux analysis in SULT4A1 expressing SH-SY5Y cells in presence or absence of H<sub>2</sub>O<sub>2</sub> to assess basal OCR, maximal OCR, spare capacity, ATP turnover and proton leak as previously described (Andrabi et al., 2014). SULT4A1 expression specifically increases maximal OCR in control SH-SY5Y cells (Fig 2 A and C). H<sub>2</sub>O<sub>2</sub> treatment in SH-SY5Y cells induces a drastic reduction in basal OCR, maximal OCR and ATP turnover (Fig 2A, B, C &D), suggesting that H<sub>2</sub>O<sub>2</sub>-mediated oxidative stress has a severe effect on mitochondrial function. SULT4A1 expression significantly increases both basal and maximal OCR in H<sub>2</sub>O<sub>2</sub>-treated SH-SY5Y cells (Fig 2A, B & C). Spare respiratory capacity also increases significantly in SH-SY5Y cells expressing SULT4A1 (Fig 2A, &E). The H<sub>2</sub>O<sub>2</sub>-induced decrease in ATP-turnover is almost reversed in SH-SY5Y cells expressing SULT4A1 (Fig 2D). Proton leak in SH-SY5Y cells expressing SULT4A1 in presence or absence of H<sub>2</sub>O<sub>2</sub> is not significantly different as compared to control (Fig 2F). The  $\Delta\Psi_m$  generated by the activities of mitochondrial electron transport chain (ETC) complexes is an important factor for oxidative phosphorylation (Murphy, 2009; Zorova et al., 2018). Loss of  $\Delta\Psi_m$  is an early feature of mitochondrial damage (Zorova et al., 2018). Mitochondria lose  $\Delta\Psi_m$  because either the ETC is not functioning correctly or the mitochondrial inner membrane proton leak (Cheng et al., 2017; Zorova et al., 2018). Nevertheless, the loss of  $\Delta\Psi_m$  is a key indicator of mitochondrial dysfunction.  $\Delta\Psi_m$  was assessed in SH-SY5Y in presence or absence of H<sub>2</sub>O<sub>2</sub> using TMRE live cell imaging with

a Zeiss LSM 710 confocal microscope (Carl Zeiss, Germany). TMRE imaging shows that H<sub>2</sub>O<sub>2</sub> almost completely abolishes  $\Delta\Psi_m$  (Fig 2G, H &I). SULT4A1 expression in these cells significantly preserved the  $\Delta\Psi_m$  (Fig 2G, H &I). These data suggest that the recovery of  $\Delta\Psi_m$  by SULT4A1 expression in SH-SY5Y cells following H<sub>2</sub>O<sub>2</sub> treatment and is not due to excessive proton leak but potentially due to direct regulatory effect on mitochondria function and redox homeostasis. We finally assessed cell viability by Alamar Blue assay 24 hours after H<sub>2</sub>O<sub>2</sub> treatment in these cells. Our results indicate H<sub>2</sub>O<sub>2</sub> induces approximately 80 % cell death in SH-SY5Y cells (Fig 2J), and SULT4A1 expression in these cells significantly protects against H<sub>2</sub>O<sub>2</sub>-induced cell death (Fig 2J). Since SH-SY5Y cells do not express detectable levels of SULT4A1 protein, we expressed SULT4A1 using lentiviral transduction. It is important to identify the role of endogenous SULT4A1 using neurons to validate the role of SULT4A1 is regulating oxidative stress. Nevertheless, the results of this study strongly indicate that SULT4A1 may have a protective role against oxidative stress in neurons.

Mitochondrial function is critical to meet high bioenergetic demands in neurons as defects in mitochondrial function lead to neurodegeneration (Gan et al., 2018; Zhao et al., 2019). Mutations in mitochondria related genes often result in neurological defects (Reeve et al., 2008; Keogh and Chinnery, 2015) and these defects are similar to those observed in the SULT4A1 KO mice. It is likely that SULT4A1 is required at neural mitochondria to meet high-energy demands, and possibly regulate ROS generated by the mitochondria (Murphy, 2009). Our data suggest that there is a functional link between SULT4A1, mitochondria and oxidative stress. How these functions of SULT4A1 are mediated, remain unknown. Because of the importance of SULT4A1 in the brain (Garcia et al., 2018), elucidation of mechanisms and pathways that SULT4A1 uses to impart its essential functions in neurons is crucial. Further experiments assessing the identification of SULT4A1-protein interaction network will provide important

insights into the roles of SULT4A1 in regulating mitochondrial function, redox homeostasis and other neuron-specific processes.

## **Authorship Contributions**

*Participated in research design:* Hossain, Marcus, Lee, Garcia and Andrabi.

*Conducted experiments:* Hossain, Marcus.

*Performed data analysis:* Hossain, Marcus, Lee, Garcia, Gagné, Poirier, Falany and Andrabi.

*Wrote or contributed to the writing of the manuscript:* Hossain, Marcus, Lee, Garcia, Gagné, Poirier, Falany and Andrabi.

## References:

- Allali-Hassani A, Pan PW, Dombrowski L, Najmanovich R, Tempel W, Dong A, Loppnau P, Martin F, Thornton J, Edwards AM, Bochkarev A, Plotnikov AN, Vedadi M, and Arrowsmith CH (2007) Structural and chemical profiling of the human cytosolic sulfotransferases. *PLoS biology* **5**:e97.
- Andrabi SA, Umanah GK, Chang C, Stevens DA, Karuppagounder SS, Gagne JP, Poirier GG, Dawson VL, and Dawson TM (2014) Poly(ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis. *Proc Natl Acad Sci U S A* **111**:10209-10214.
- Cheng J, Nanayakkara G, Shao Y, Cueto R, Wang L, Yang WY, Tian Y, Wang H, and Yang X (2017) Mitochondrial Proton Leak Plays a Critical Role in Pathogenesis of Cardiovascular Diseases. *Adv Exp Med Biol* **982**:359-370.
- Crittenden F, Thomas HR, Parant JM, and Falany CN (2015) Activity Suppression Behavior Phenotype in SULT4A1 Frameshift Mutant Zebrafish. *Drug Metab Dispos* **43**:1037-1044.
- Dimauro I, Pearson T, Caporossi D, and Jackson MJ (2012) A simple protocol for the subcellular fractionation of skeletal muscle cells and tissue. *BMC research notes* **5**:513.
- Falany CN, Xie X, Wang J, Ferrer J, and Falany JL (2000) Molecular cloning and expression of novel sulphotransferase-like cDNAs from human and rat brain. *Biochem J* **346 Pt 3**:857-864.
- Gan L, Cookson MR, Petrucelli L, and La Spada AR (2018) Converging pathways in neurodegeneration, from genetics to mechanisms. *Nat Neurosci* **21**:1300-1309.
- Garcia PL, Hossain MI, Andrabi SA, and Falany CN (2018) Generation and Characterization of SULT4A1 Mutant Mouse Models. *Drug Metab Dispos* **46**:41-45.
- Hossain MI, Roulston CL, Kamaruddin MA, Chu PW, Ng DC, Disting GJ, Bjorge JD, Williamson NA, Fujita DJ, Cheung SN, Chan TO, Hill AF, and Cheng HC (2013) A truncated fragment of Src protein kinase generated by calpain-mediated cleavage is a mediator of neuronal death in excitotoxicity. *J Biol Chem* **288**:9696-9709.
- Keogh MJ and Chinnery PF (2015) Mitochondrial DNA mutations in neurodegeneration. *Biochim Biophys Acta* **1847**:1401-1411.
- Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* **417**:1-13.

- Nohl H, Gille L, and Staniek K (2005) Intracellular generation of reactive oxygen species by mitochondria. *Biochem Pharmacol* **69**:719-723.
- Raichle ME and Gusnard DA (2002) Appraising the brain's energy budget. *Proc Natl Acad Sci U S A* **99**:10237-10239.
- Reeve AK, Krishnan KJ, and Turnbull D (2008) Mitochondrial DNA mutations in disease, aging, and neurodegeneration. *Ann N Y Acad Sci* **1147**:21-29.
- Sidharthan NP, Butcher NJ, Mitchell DJ, and Minchin RF (2014) Expression of the orphan cytosolic sulfotransferase SULT4A1 and its major splice variant in human tissues and cells: dimerization, degradation and polyubiquitination. *PloS one* **9**:e101520.
- Suiko M, Kurogi K, Hashiguchi T, Sakakibara Y, and Liu MC (2017) Updated perspectives on the cytosolic sulfotransferases (SULTs) and SULT-mediated sulfation. *Biosci Biotechnol Biochem* **81**:63-72.
- Wang W, Wang L, Lu J, Siedlak SL, Fujioka H, Liang J, Jiang S, Ma X, Jiang Z, da Rocha EL, Sheng M, Choi H, Lerou PH, Li H, and Wang X (2016) The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. *Nature medicine* **22**:869-878.
- Wazawa T, Matsuoka A, Tajima G, Sugawara Y, Nakamura K, and Shikama K (1992) Hydrogen peroxide plays a key role in the oxidation reaction of myoglobin by molecular oxygen. A computer simulation. *Biophys J* **63**:544-550.
- Zhao XY, Lu MH, Yuan DJ, Xu DE, Yao PP, Ji WL, Chen H, Liu WL, Yan CX, Xia YY, Li S, Tao J, and Ma QH (2019) Mitochondrial Dysfunction in Neural Injury. *Front Neurosci* **13**:30.
- Zorova LD, Popkov VA, Plotnikov EY, Silachev DN, Pevzner IB, Jankauskas SS, Babenko VA, Zorov SD, Balakireva AV, Juhaszova M, Sollott SJ, and Zorov DB (2018) Mitochondrial membrane potential. *Anal Biochem* **552**:50-59.



**Footnotes:**

This work was supported by the National Institute of Health/National Institute of Neurological Disorders and Stroke [Grant NS086953].

**Deceleration of Interest:**

The authors declare no competing interests.

## Figure Legend

**Fig 1.** (A) Western blot showing knockdown of SULT4A1 in primary neurons. (B) CellRox fluorescence in primary cortical neurons transduced with control shRNA or SULT4A1 shRNA. Scale bar = 20  $\mu$ m. (C) Quantification of CellRox fluorescence intensity. Data represents mean  $\pm$  SEM,  $n = 5$ , \*\*\* $P < 0.001$  vs. control (Student's t-test). (D) Western blots showing SULT4A1 expression in SH-SY5Y cells with or without transduction with control virus or SULT4A1 lentivirus. (E) Subcellular fractionation in SH-SY5Y showing mitochondrial localization of SULT4A1. TOM20 is used as a mitochondrial marker and GAPDH is cytosolic marker. (F) Immunostaining pictures of SULT4A1 with orthogonal projections in SH-SY5Y show that SULT4A1 (green) localize with mitochondrial marker TOM20 (red). Co-localization was determined by Pearson's correlation coefficient,  $R = 0.66$ .

**Fig 2.** Mitochondrial function in SH-SY5Y cells expressing SULT4A1. (A) Representative data showing mitochondrial OCR in SH-SY5Y cells transduced with control or SULT4A1 virus in presence or absence of  $H_2O_2$ . Quantification of Basal respiration (B), Maximal respiration (C), ATP turnover (D), Spare respiration capacity (E) and Proton leak (F). Data is mean  $\pm$  SEM,  $n = 18-22$  for each group. Experiments were repeated 3 times with similar results. (G) Confocal images showing TMRE intensity in SH-SY5Y cells transduced with control and SULT4A1 virus in presence or absence of  $H_2O_2$ , Scale bar = 20  $\mu$ m. (H) Time-lapse quantification of TMRE fluorescence intensity before and after CCCP addition. (I) Bar graph showing quantification of TMRE intensity change ( $\Delta$ TMRE) before and after CCCP addition in SH-SY5Y cells. Data represents mean  $\pm$  SEM,  $n = 3$ . (J) Cell viability in SH-SY5Y cells in presence or absence of  $H_2O_2$ . Data is mean  $\pm$  SEM,  $n = 8$ . \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs control, was calculated using one-way ANOVA and Tukey's post hoc test.

Figure 1

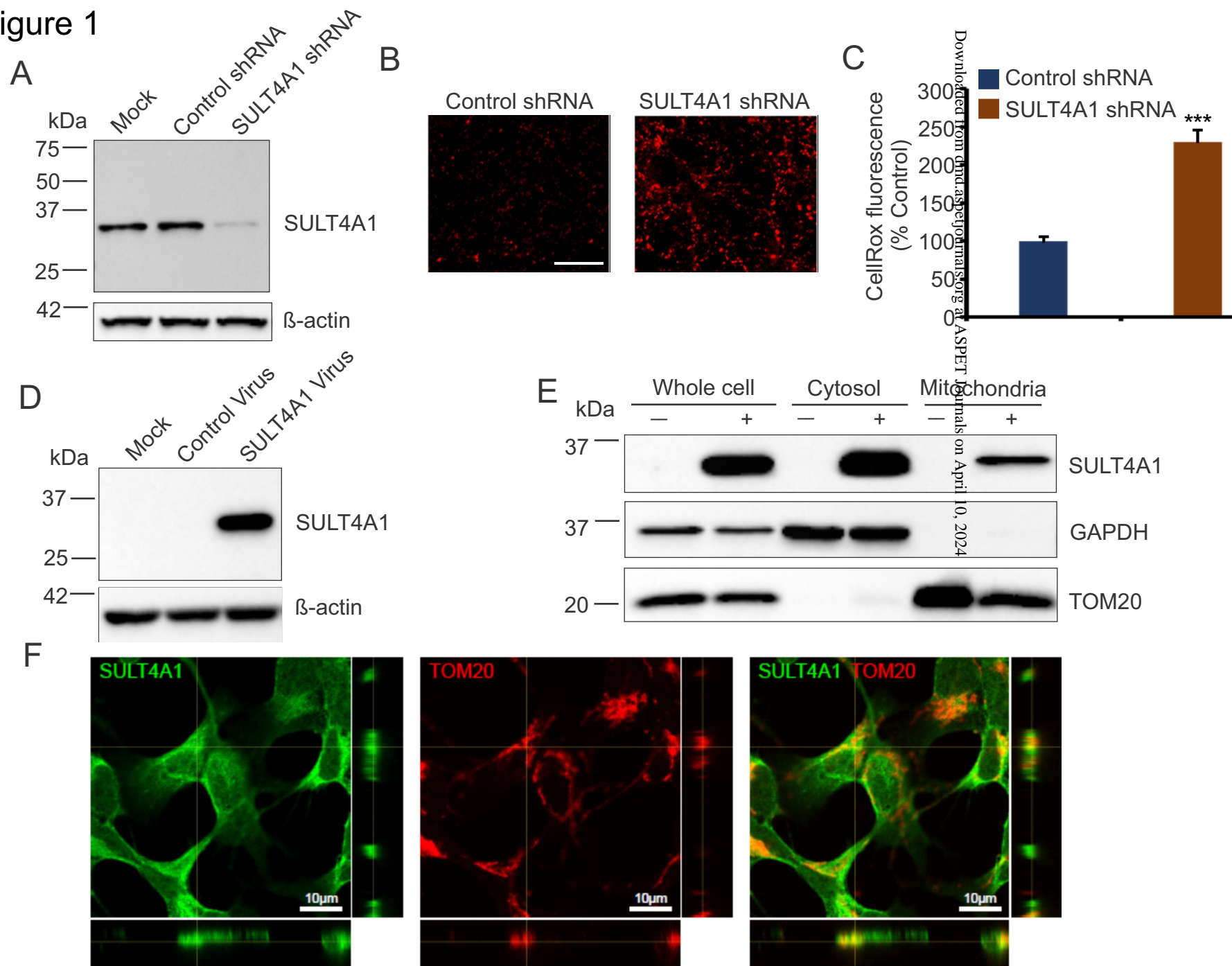


Figure 2

