Supplement to

Clinical and functional consequences of C-terminal variants in MCT8

Ferdy S. van Geest [1], Marcel E. Meima [1], Kyra E. Stuurman [2], Nicole I. Wolf [3,4], Marjo S. van der Knaap [3,4], Cláudia F. Lorea [5], Fabiano O. Poswar [6], Filippo Vairo [7], Nicola Brunetti-Pierri [8,9], Gerarda Cappuccio [8,9], Priyanka Bakhtiani [10], Sonja A. de Munnik [11], Robin P. Peeters [1], W. Edward Visser [1], Stefan Groeneweg [1]

[1] The Rotterdam Thyroid Center & Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; [2] Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; [3] Department of Pediatric Neurology, Emma Children's Hospital, Amsterdam University Medical Centre, Amsterdam, Netherlands; [4] Amsterdam Neuroscience, Amsterdam, Netherlands; [5] Teaching Hospital of Universidade Federal de Pelotas, Brazil; [6] Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; [7] Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA, 55905; Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA, 55905; [8] Department of Translational Medicine, Federico II University, 80131 Naples, Italy; [9] Telethon Institute of Genetics and Medicine, Pozzuoli, 80078 Naples, Italy; [10] University of Louisville, Kentucky, USA; [11] Department of Human Genetics, Radboud University Medical Centre Nijmegen, Nijmegen, the Netherlands.

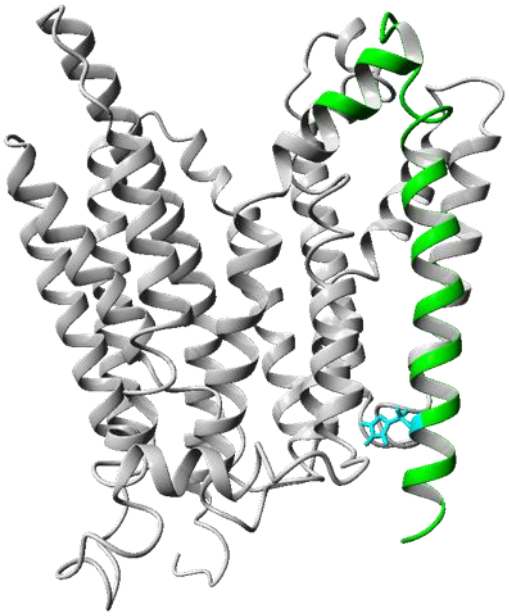
Supplemental Tables

Supplemental Table S1 Antibody Table

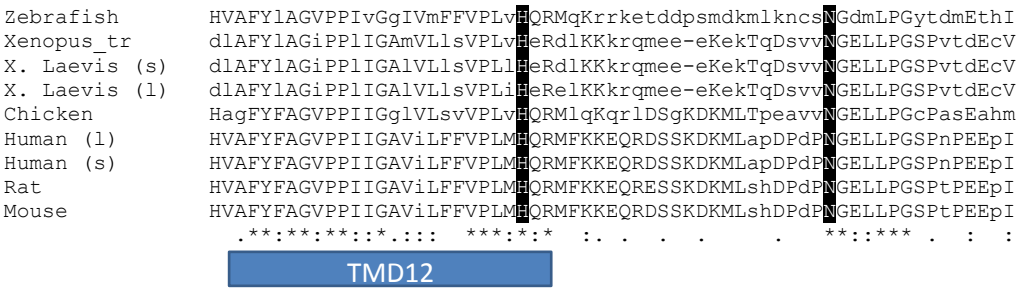
Target protein/ antigen	Antigen sequence (if known)	Name of AB	Species raised (P or M)	Manufacturer (and catalog number)	Dilution used for WB	Dilution used for ICH	RRID
hsMCT8	AA 52-155	MCT8	Rabbit (P)	ATLAS (HPA003353)	1:2000	1:1000	AB_1079343
GAPDH		GAPDH	Mouse (M)	Millipore (Mab 374)	1:20000		AB_2107445
ZO1		ZO1	Mouse (M)	Thermo Fisher (33-9100)		1:1000	AB_2533147
Rabbit IgG		IRDye800	Goat	LI-COR (926-32211)	1:20000		AB_621843
Mouse IgG		IRDye680	Goat	LI-COR (926-68020)	1:20000		AB_10706161
Rabbit IgG		Alexa 488	Goat	Thermo Fisher (A11008)		1:1000	AB_143165
Mouse IgG		Alexa 633	Goat	Thermo Fisher (A21050)		1:1000	AB_2535718

AB: antibody; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; ZO-1: zona occludens 1;P: polyclonal antibody; M: monoclonal antibody; WB: Western Blot ; ICH: immunohistochemistry

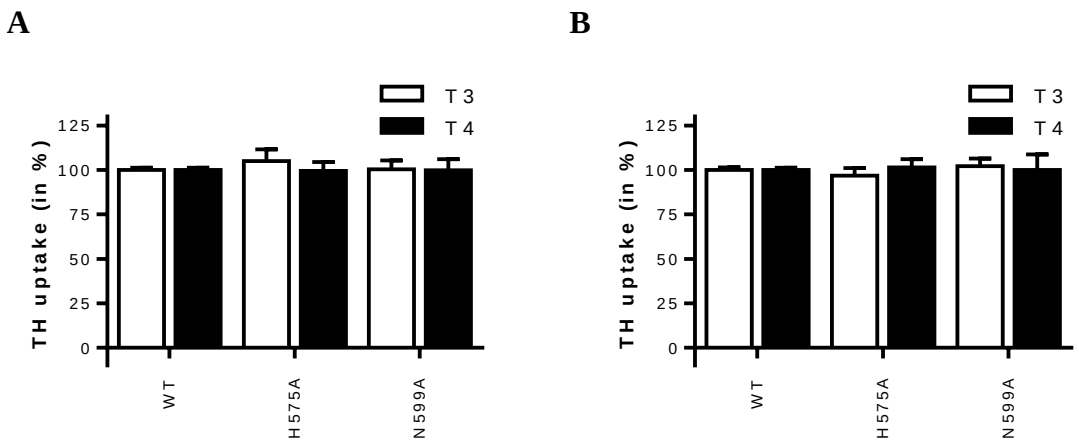
Supplemental Figures



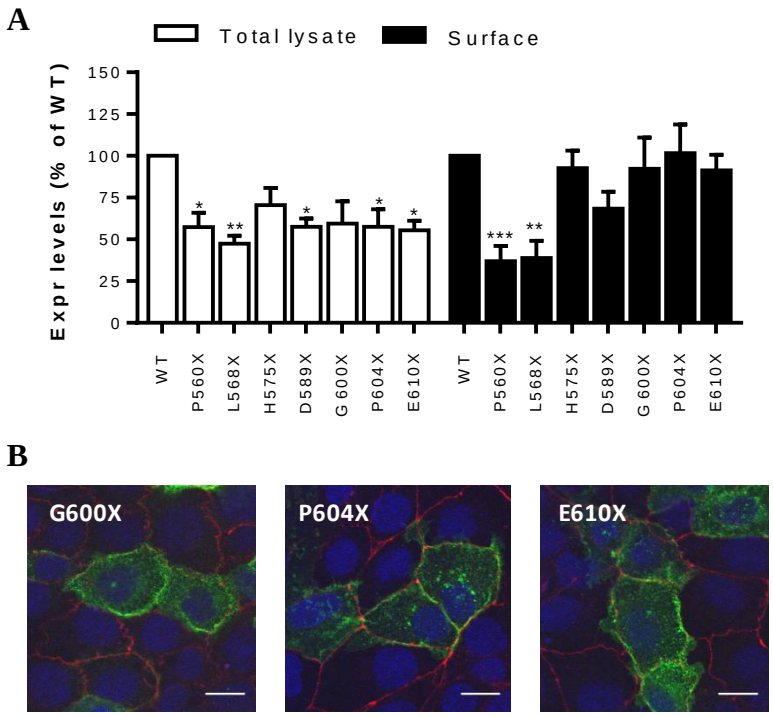
Supplemental Figure S1 MCT8 homology model in which the residues encoded by exon 6 are highlighted in green. The His575 residue is highlighted in blue. The Asn599 residue is not included in the currently available MCT8 homology models due to poor sequence alignment between the intracellular C-terminal tail of MCT8 and the available template structures.



Supplemental Figure S2 Multiple sequence alignment of the C-terminal end of the MCT8 protein. This region includes transmembrane domain (TMD)12 and the intracellular C-terminal tail (see Figure 1A and B). Amino acid sequences of human, mouse, zebrafish, *Xenopus Tropicalis* (Xenopus tr), the short (s) and long (l) isoforms of *Xenopus laevis* (X. Laevis), chicken, and rat were included. Identical amino acids in all species are indicated with an *. Conservation between amino acids with strongly similar properties (equivalent to scoring > 0.5 in the Gonnet PAM 250 matrix) is indicated with an :, whereas conservation between amino acids with weakly similar properties (equivalent to scoring 0< score <0.5 in the Gonnet PAM 250 matrix) are indicated with a . . Multiple sequence alignments were generated using Clustal Omega. The His575 and Asn599 residues in human MCT8 and their corresponding (His and Asn) residues in other species are highlighted in black.



Supplemental Figure S3 T3 and T4 uptake in transiently transfected COS-1 **(A)** or JEG-3 **(B)** cells in presence of CRYM, after 30 min incubation at 37 °C. Uptake levels are corrected for those observed in pcDNA3 empty vector (EV) transfected control cells and expressed relative to wild-type (WT) MCT8. Data are presented as means ± SEM based on at least 3 independent experiments in duplicate. Two-way ANOVA with Bonferroni post-tests were performed to assess for statistically significant differences between WT and indicated MCT8 variants.



Supplemental Figure S4 (A) Quantification of WT and indicated mutant MCT8 protein in total lysates and the biotinylated cell surface fraction in transiently transfected COS-1 cells (representative blots are shown in Figure 4E and 4F). Expression levels were quantified using ImageJ software and expressed relative to WT MCT8. The means ± SEM from N=3 independent experiments are displayed and compared to WT MCT8 expression levels using Two-way ANOVA with Bonferroni post-tests. Statistically significant differences are indicated as follows: $p<0.05$,*; $p<0.01$, **; $p<0.001$, ***. **(B)** Immunocytochemistry in JEG-3 cells transiently transfected with indicated MCT8 variants using antibodies against MCT8 (green) and the membrane marker ZO-1 (red). Cell nuclei were stained with DAPI (blue). Images are presented as an overlay image. The scale bar represents 20µm.