**Supplemental document to:**

**Insights into the mechanism of MCT8 oligomerization**

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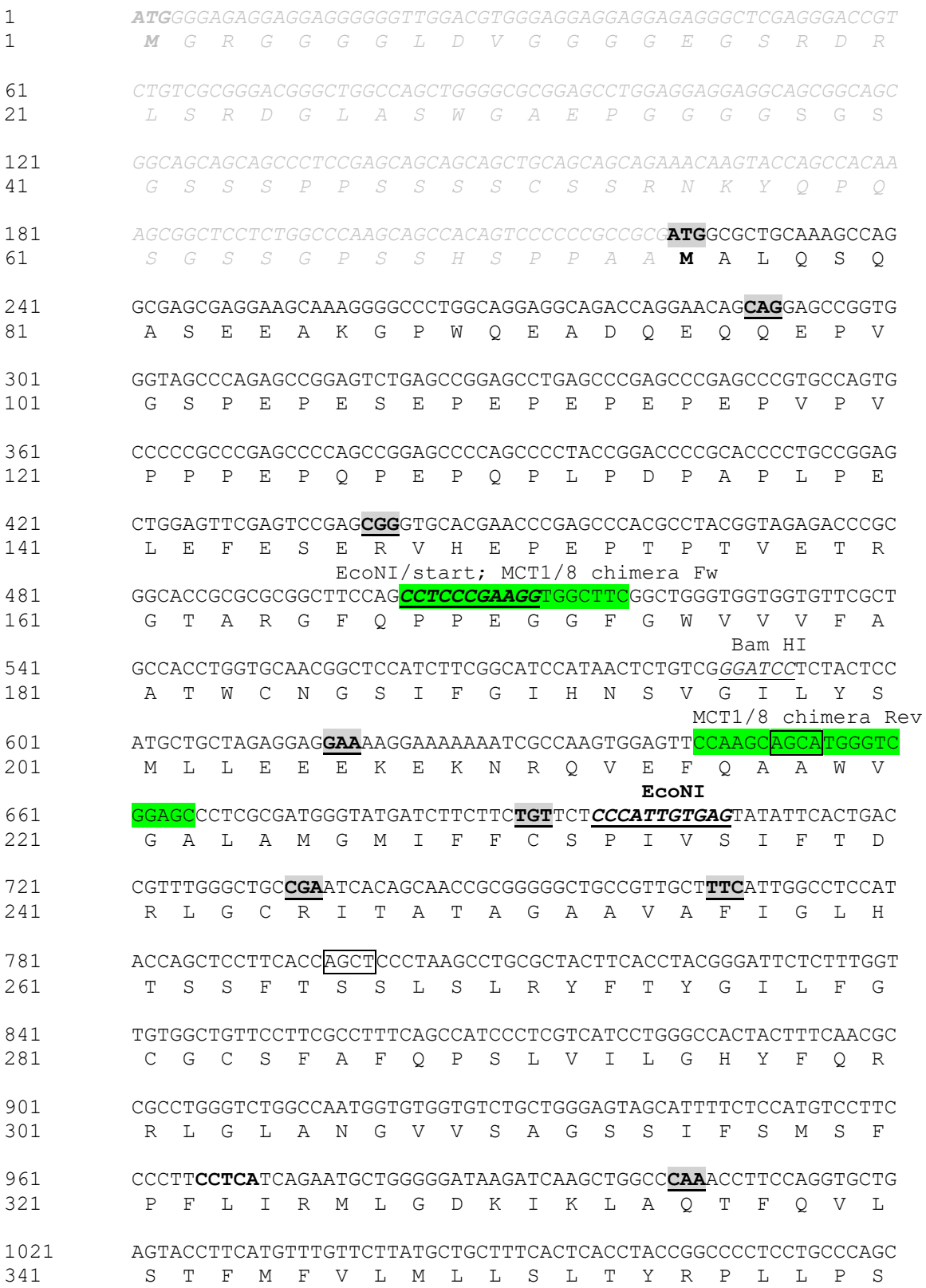
***Abbreviated Title:*** MCT8 oligomerization

**Supplemental Fig. 1**



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1081      TCCCAGGACACCCCAAGCAAGAGAGGTGTCCGCACCCTGCACCAGCGCTTTCTGGCTCAG
361        S  Q  D  T  P  S  K  R  G  V  R  T  L  H  Q  R  F  L  A  Q


1141      CTCAGGAAGTACTTCAACATGCGAGTGTTCCGCCAACGCACTTACCGCATCTGGGCCTTC
381        L  R  K  Y  F  N  M  R  V  F  R  Q  R  T  Y  R  I  W  A  F

1201      GGAATTGCTGCTGCTGCCCTTGGCTACTTTGTTCCCTATGTACACCTGATGAAGTATGTG
401        G  I  A  A  A  A  L  G  Y  F  V  P  Y  V  H  L  M  K  Y  V

1261      GAGGAGGAGTTCTCAGAAATCAAGGAGACCTGGGTGCTCTTGGTGTGTATTGGGGCTACC
421        E  E  E  F  S  E  I  K  E  T  W  V  L  L  V  C  I  G  A  T

1321      TCAGGCCTTGGGCGTCTTGTGTCAGGCCACATCAGTGACTCCATCCCTGGACTTAAGAAG
441        S  G  L  G  R  L  V  S  G  H  I  S  D  S  I  P  G  L  K  K

1381      ATCTACTTGCAGGTCCTTTCCTTCCTGCTCCTGGGCCTGATGTCCATGATGATTCCCCTG
461        I  Y  L  Q  V  L  S  F  L  L  L  G  L  M  S  M  M  I  P  L

1441      TGCCGGGACTTCGGGGGCCTTATCGTCGTCTGTCTTTTCCTGGGCCTTTGCGATGGCTTC
481        C  R  D  F  G  G  L  I  V  V  C  L  F  L  G  L  C  D  G  F

1501      TTCATCACCATCATGGCCCCCATTGCATTTGAGCTGGTGGGCCCAATGCAGGCCTCACAG
501        F  I  T  I  M  A  P  I  A  F  E  L  V  G  P  M  Q  A  S  Q

1561      GCCATTGGCTACCTCCTGGGCATGATGGCCCTGCCAATGATTGCTGGGCCCCCCATTGCA
521        A  I  G  Y  L  L  G  M  M  A  L  P  M  I  A  G  P  P  I  A

1621      GGCCTACTCCGCAACTGTTTTGGGGACTACCATGTGGCCTTCTACTTTGCCGGTGTGCCC
541        G  L  L  R  N  C  F  G  D  Y  H  V  A  F  Y  F  A  G  V  P

1681      CCCATCATCGGGGCTGTAATCCTCTTCTTCGTCCCTCTGATGCATCAAAGGATGTTCAAG
561        P  I  I  G  A  V  I  L  F  F  V  P  L  M  H  Q  R  M  F  K

1741      AAAGAGCAGAGAGATTCCAGCAAGGATAAGATGTTGGCCCCTGACCCAGACCCCAATGGG
581        K  E  Q  R  D  S  S  K  D  K  M  L  A  P  D  P  D  P  N  G

1801      GAGCTACTGCCGGGCTCCCCCAACCCTGAGGAACCAATCTAA
601        E  L  L  P  G  S  P  N  P  E  E  P  I  *
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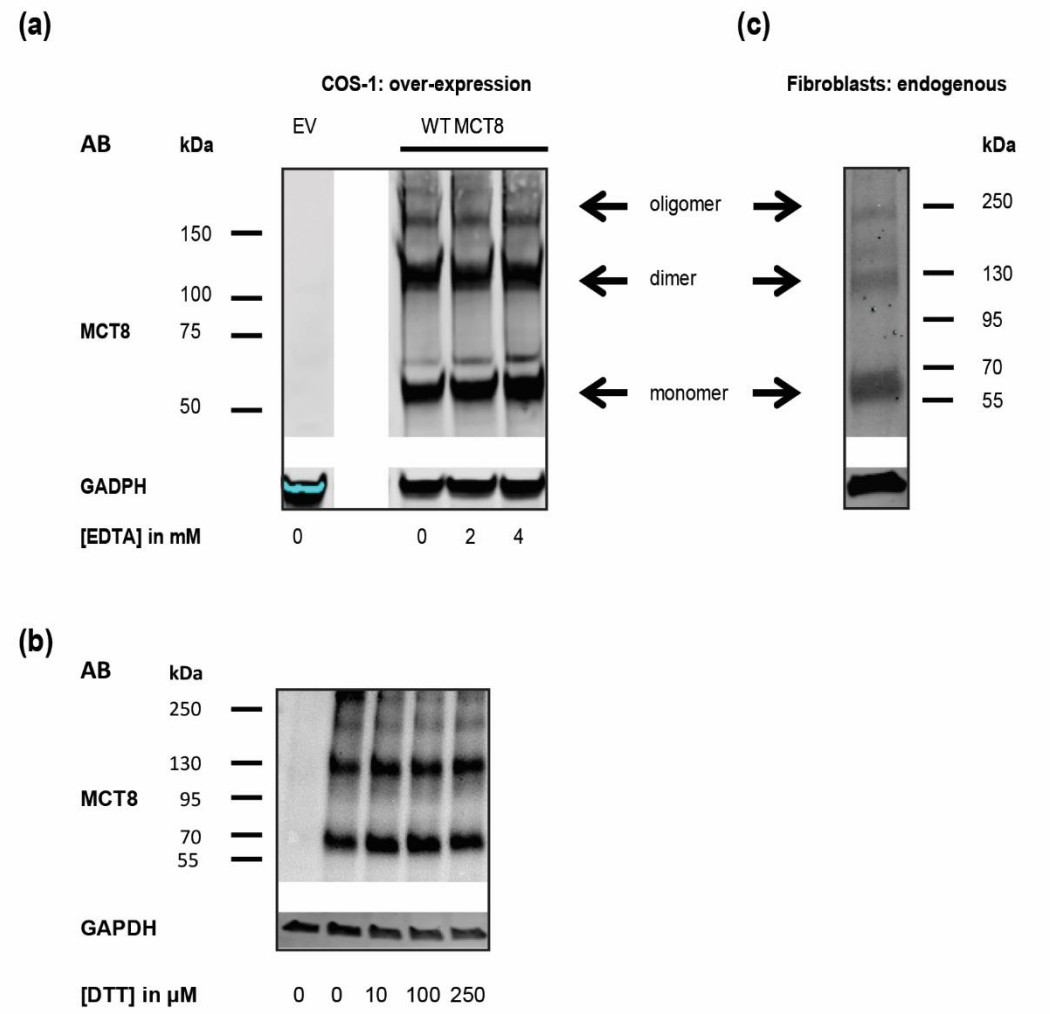
cDNA sequence of coding region of the hMCT8 construct that was used for the generation of all described mutant and tagged MCT8 constructs. This construct only contains the coding sequence of the short MCT8 isoform starting at Met75. The sequence encoding the unique part of the long MCT8 isoform is depicted in *light grey* and is shown to clarify the amino acid numbering. Locations of the introduced mutations are in bold, underlined and highlighted in grey. The Bam HI and Eco NI restriction sites are displayed in italic and underlined font, the locations of the forward and reverse primer used in the generation of the MCT1/8 chimera are highlighted in green and the splice sites are boxed.

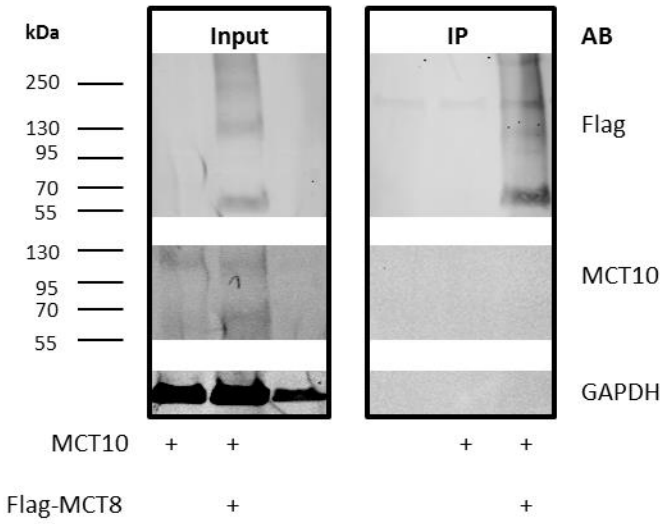
**Supplemental Figure 2**




**(A)** Immunoblot of total cell lysates derived from COS-1 cells transiently expressing WT MCT8 incubated with increasing concentrations of the Ca2+ / Mg2+ chelator EDTA for 30 minutes before the addition of LDS loading buffer, containing 10 mM DTT. Note that MCT8 is expressed as monomer (~55 kDa), homo-dimer (~110 kDa) and homo-oligomer (~170 kDa). Increasing concentrations of EDTA did not affect MCT8 oligomerization. MCT8 was detected using the N-terminal MCT8 antibody (3353) and GAPDH was used as a loading control. **(B)** Immunoblot on total lysates of COS-1 cells transiently expressing WT MCT8 incubated with increasing concentrations DTT in LDS for 10 minutes at 70°C prior to separation. Increasing concentrations of DTT did not affect MCT8 oligomerization. MCT8 was detected using the N-terminal

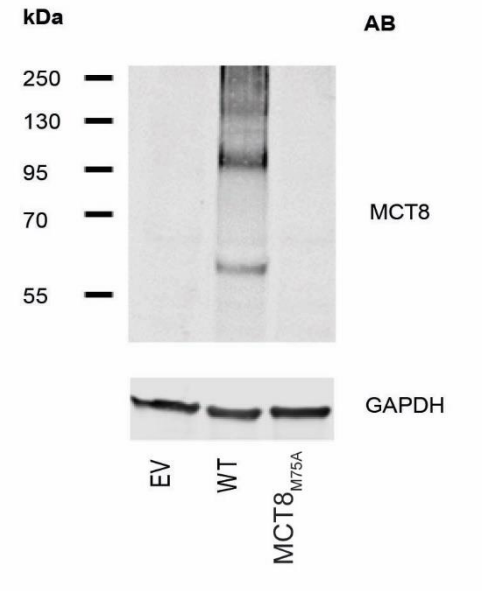
MCT8 antibody (3353) and GAPDH was used as a loading control. **(C)** Immublot on total lysates derived from skin fibroblasts obtained from a healthy subject, using the N-terminal MCT8 antibody (3353) and GAPDH as a loading control. Note that endogenously expressed MCT8 is also detected as a monomer, homo-dimer and putative homo-oligomer. Abbreviations: EDTA, Ethylenediaminetetraacetic acid; EV, empty vector.

**Supplemental Figure 3**



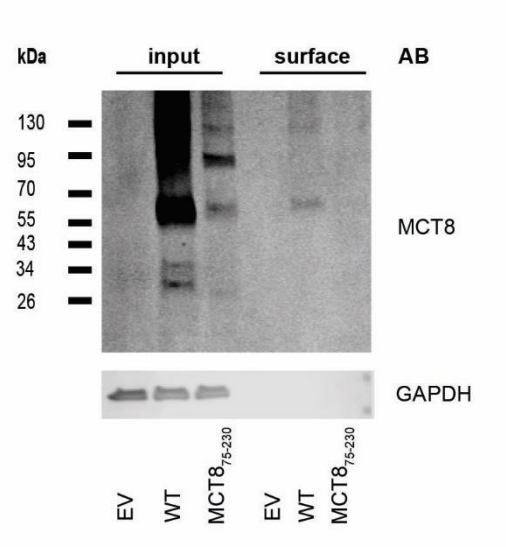

Representative blots (N=3) of co-IP studies on lysates derived from COS-1 cells transiently expressing MCT10 alone (lane 2), or in combination with Flag-MCT8 (lane3). Blots were simultaneously stained with mouse M2 anti-Flag and rabbit anti-MCT10 antiserum (designated 1758). MCT10 could not be enriched by Flag-MCT8 mediated IPs. Abbreviations: AB, antibody; IP, immunoprecipitation.

**Supplemental Figure 4**



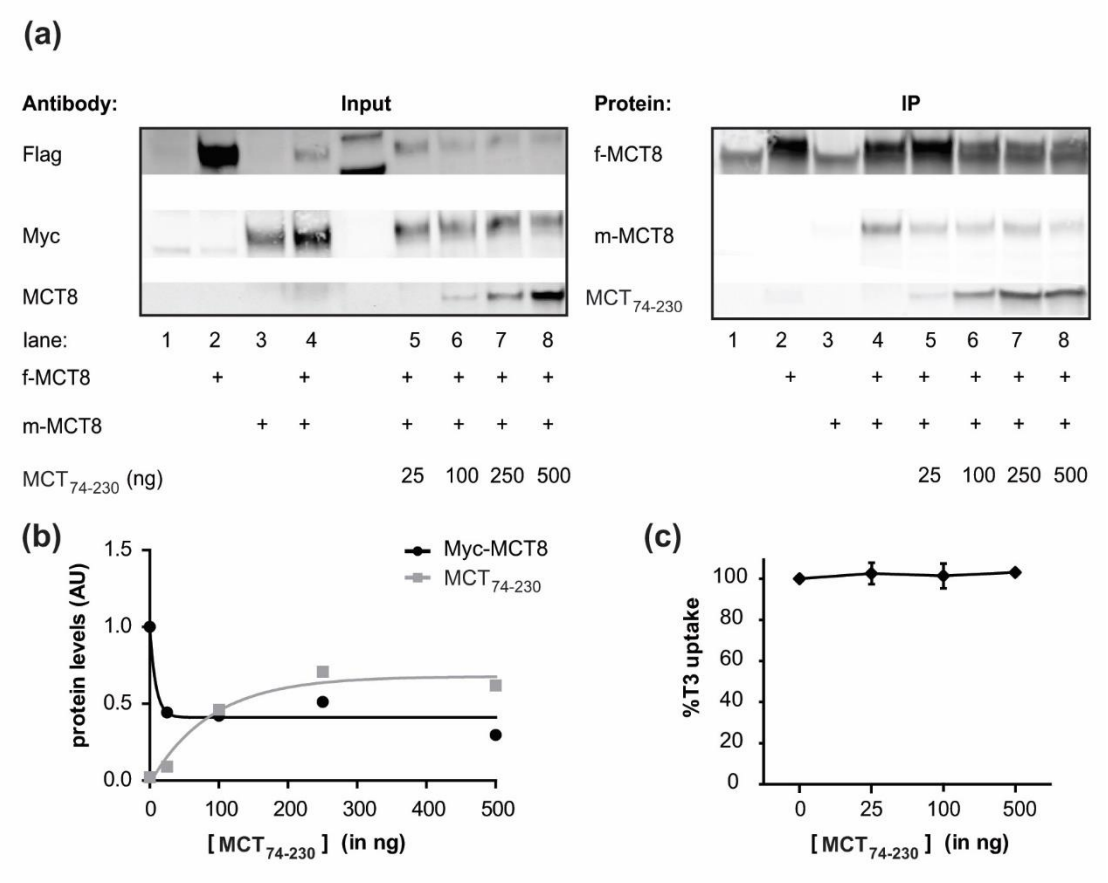

Representative immunoblot of lysates from COS-1 cells transfected with WT MCT8 or the $MCT8_{M75A}$ mutant (N=2), performed as described in Fig. 2D. Note, that no signal was obtained upon inactivation of the natural translation start site (Met75) using antibodies directed against the N-terminus of MCT8. Abbreviations: AB, antibody; EV, empty vector.

**Supplemental Figure 5**




Representative Western Blot analyses on total lysates and the cell surface fraction derived from COS-1 cells transfected with pcDNA3 EV, WT MCT8 or the $MCT8_{74-230}$ mutant. The input sample comprises 5% of the clarified lysate from which the presented surface fraction was derived. MCT8 detection was performed with the N-terminal MCT8 antibody (3353) and visualized with IRDye680 goat anti-rabbit secondary antibody. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. Note the absence of GAPDH in the cell surface samples, demonstrating the purity of the cell surface fraction. Whereas bands corresponding to WT monomer and dimer were detected in the surface fraction, no bands corresponding to the $MCT8_{74-230}$ mutant MCT8 were observed, confirming the confocal data presented in Figure 2C. Abbreviations: AB, antibody; EV, empty vector; WT, wild-type.

**Supplemental Figure 6**




Representative blots of co-IP studies on lysates derived from COS-1 cells transiently expressing Flag-MCT8 (75 ng cDNA, lane 2), Myc-MCT8 (75 ng cDNA, lane 3) alone, or combined (lane 4), and COS-1 cells co-expressing Flag-MCT8 and Myc-MCT8 and increasing amounts of the $MCT8_{75-230}$ mutant construct (lane 5-8). Blots were subsequently stained with mouse M2 anti-Flag antibody and rabbit anti-Myc antibody, stripped and stained with rabbit anti-MCT8 antibody. Input and IP samples were run on separate gels (boxed). Note that increasing concentrations of $MCT8_{75-230}$ reduced the amount of Myc-MCT8 detected in the IP samples, indicating that $MCT8_{75-230}$ may compete with the formation of WT Flag-MCT8/Myc-MCT8 interactions. Please note the decrease in Flag-MCT8 signal on the input blot with increasing concentrations of $MCT8_{75-230}$ **(B)** Quantification by densitometry (using ImageJ) of the Myc-MCT8 and $MCT8_{75-230}$ protein levels in the IP samples shown in **(A),** expressed relative to the intensity of the Flag-MCT8 band in the same

IP sample. **(C)** Parallel T3 uptake studies (30 minutes at 37 C) in COS-1 cells co-expressing equal amounts of WT Flag-MCT8 and Myc-MCT8 as in **(A)**, increasing amounts of the $MCT8_{75-230}$ mutant, and a fixed amount of CRYM (250 ng). The results of the uptake studies are presented as means ± SEM (n=3). There are no statistically significant differences as analyzed with a 1-way ANOVA followed by Dunnett's multiple-comparison test. Abbreviations: AU, arbitrary units.

**Supplemental Table 1**

**Antibody Table**

| Target protein/ antigen | Antigen Sequence (if known) | Name of Antibody | Species Raised (polyclonal or monoclonal) | Manufacturer (and catalog number) | Dilution used for WB | Dilution used for ICH | RRID |
|---|---|---|---|---|---|---|---|
| hMCT8 | AA 52-155 N-terminus | MCT8 (3353) | Rabbit (p) | ATLAS (HPA003353) | 1:20000 | 1:1000 | AB_1079343 (1) |
| hMCT8 | AA 527–539 C-terminus | MCT8 (1306) | Rabbit (p) | Personal AB (19) | 1:1000 | 1:500 | AB_2661880 (2) |
| hMCT10 | AA 473–487 and 503–515 | MCT10 (1758) | Rabbit (p) | Personal AB (19) | 1:1000 | | AB_2661879 (3) |
| GAPDH | | GAPDH | Mouse (m) | Millipore (Mab 374) | 1:20000 | | AB_2107445 (4) |
| ZO1 | | ZO1 | Mouse (m) | Thermo Fisher (33-9100) | 1:1000 | 1:1000 | AB_2533147 (5) |
| Flag | Flag epitope | Flag-M2 | Mouse (m) | Sigma (C3956) | 1:1000 | | AB_262044 (6) |
| Myc | Myc epitope | Myc | Rabbit (m) | Sigma (F1804) | 1:1000 | | AB_439680 (7) |
| Mouse IgG | | IRDye680 | Goat | LI-COR (926-68020) | 1:20000 | | AB_10706161 (8) |
| Rabbit IgG | | IRDye800 | Goat | LI-COR (926-32211) | 1:20000 | | AB_621843 (9) |
| Rabbit IgG | | Alexa 488 | Goat | Thermo Fisher (A11008) | | 1:1000 | AB_143165 (10) |
| Mouse IgG | | Alexa 633 | Goat | Thermo Fisher (A21050) | | 1:1000 | AB_2535718 (11) |

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

**Supplemental Table 2**

| **Overview of primers** | |
|---|---|
| **Name** | **sequence from 5' to 3'** |
| E206X_Fw | CTCCATGCTGCTAGAGGAG**TAA**AAGGAAAAAAATCGC |
| E206X_Rev | GCGATTTTTTTCCTTTTACTCCTCTAGCAGCATGGAG |
| C231X_Fw | GATGGGTATGATCTTCTTC**TGA**TCTCCCATTGTGAGTATATTC |
| C231X_Rev | GAATATACTCACAATGGGAGATCAGAAGAAGATCATACCCATC |
| R245X_Fw | GACCGTTTGGGCTGC**TGA**ATCACAGCAACCG |
| R245X_Rev | CGGTTGCTGTGATTCAGCAGCCCAAACGGTC |
| F256X_Fw | GGGCTGCCGTTGCT**TAA**ATTGGCCTCCATACCAG |
| F256X_Rev | CTGGTATGGAGGCCAATTTAAGCAACGGCAGCCC |
| Q335X_Fw | ATAAGATCAAGCTGGCC**TAA**ACCTTCCAGGTGCTG |
| Q335X_Rev | CAGCACCTGGAAGGTTTAGGCCAGCTTGATCTTAT |
| S448X_Fw | GGCCTTGGGCGTCTTGTG**TAA**GGCCACATC |
| S448X_Rev | GATGTGGCCTTACACAAGACGCCCAAGGCC |
| Q520X_Fw | GGGCCCAATGCAGGCCTCA**TAG**GCCATTGG |
| Q520X_Rev | CCAATGGCCTATGAGGCCTGCATTGGGCCC |
| C546X_Fw | GCCTACTCCGCAAC**TGA**TTTGGGGACTACCATG |
| C546X_Rev | CATGGTAGTCCCCAAATCAGTTGCGGAGTAGGC |
| M74A_Fw | CAGTCCCCCCGCCGCG**GCTT**CGCTGCAAAGCCAGGC |
| M74A_Rev | GCCTGGCTTTGCAGCGAAGCCGCGGCGGGGGGACTG |
| Q97M_Fw | CTGGCAGGAGGCAGACCAGGAA**GCGATGGCG**CCGGTGGGTAG |
| Q97M_Rev | CTACCCACCGGCGCCATCGCTTCCTGGTCTGCCTCCTGCCAG |
| R147M_Fw | GAGTTCGAGTCCGAG**ATGGCG**CACGAACCCGAGCC |
| R147M_Rev | GGCTCGGGTTCGTGCGCCATCTCGGACTCGAACTC |
| MCT8 Eco NI_Fw | GGGTATGATCTTCTTCTGTTCTCCTATCGTAGGTATATTCACTGACCGTTTGGGCT |
| MCT8 Eco NI_Rev | AGCCCAAACGGTCAGTGAATATACCTACGATAGGAGAACAGAAGAAGATCATACCC |
| MCT1_Fw | CGTATTAAGCTTACTGGTCGGTCCTGTAGGTG |
| MCT1_Rev | GTATAAGCTAGCGGTGTATCCCACTGGCC |
| MCT8_Fw | CATATTGCTAGCCCTCCCGAAGGTGGCTTC |
| MCT8_Rev | GCTCCGACCCATGCTGCTTGG |
| Flag_MCT8_Fw | GATAAGCTTCAGAAATGGACTACAAAGACGATGACGACAAGGCGCTGCAAAGCCAGG |
| Flag_MCT8_Rev | ATCTCTAGATTAGATTGGTTCCTCAGGGTTGGG |
| Myc_MCT8_Fw | GATAAGCTTCAGAAATGGAACAAAAACTCATCTCAGAAGAGGATCTGGCGCTGCAAAGCCAGG |
| Myc_MCT8_Rev | ATCTCTAGATTAGATTGGTTCCTCAGGGTTGGG |
| *Restriction sites within the primers are underlined; the locations of introduced mutations are depicted in bold.* | |

**References**


1. RRID:AB_1079343, https://scicrunch.org/resolver/AB_1079343
2. RRID:AB_2661880, https://scicrunch.org/resolver/AB_2661880
3. RRID:AB_2661879, https://scicrunch.org/resolver/AB_2661879
4. RRID:AB_2107445, https://scicrunch.org/resolver/AB_2107445
5. RRID:AB_2533147, https://scicrunch.org/resolver/AB_2533147
6. RRID:AB_262044, https://scicrunch.org/resolver/AB_262044
7. RRID:AB_439680, https://scicrunch.org/resolver/AB_439680
8. RRID:AB_10706161, https://scicrunch.org/resolver/AB_10706161
9. RRID:AB_621843, https://scicrunch.org/resolver/AB_621843
10. RRID:AB_143165, https://scicrunch.org/resolver/AB_143165
11. RRID:AB_2535718, https://scicrunch.org/resolver/AB_2535718