Supplemental Materials to:

Insights into the mechanism of MCT8 oligomerization

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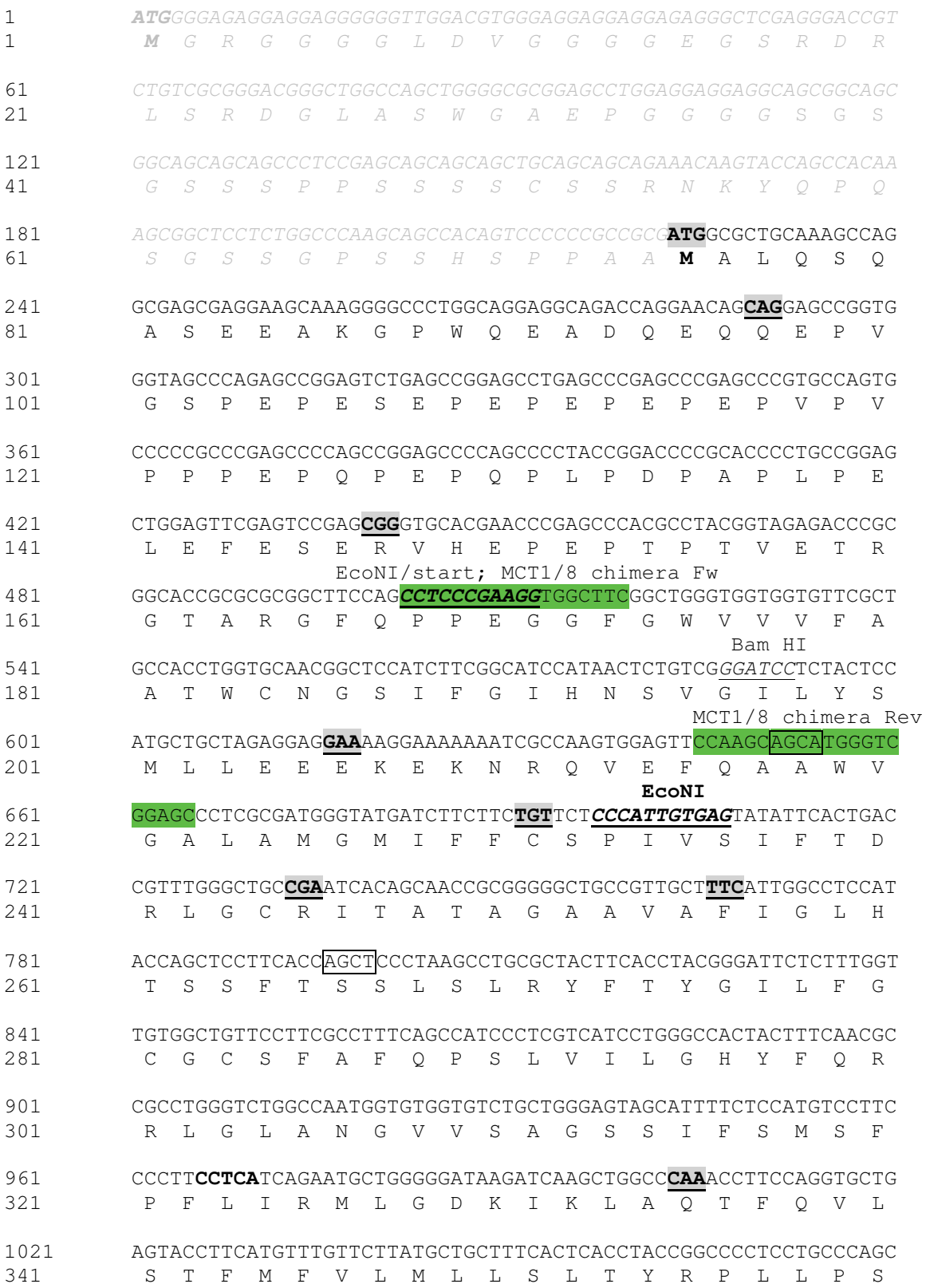
Abbreviated Title: MCT8 oligomerization

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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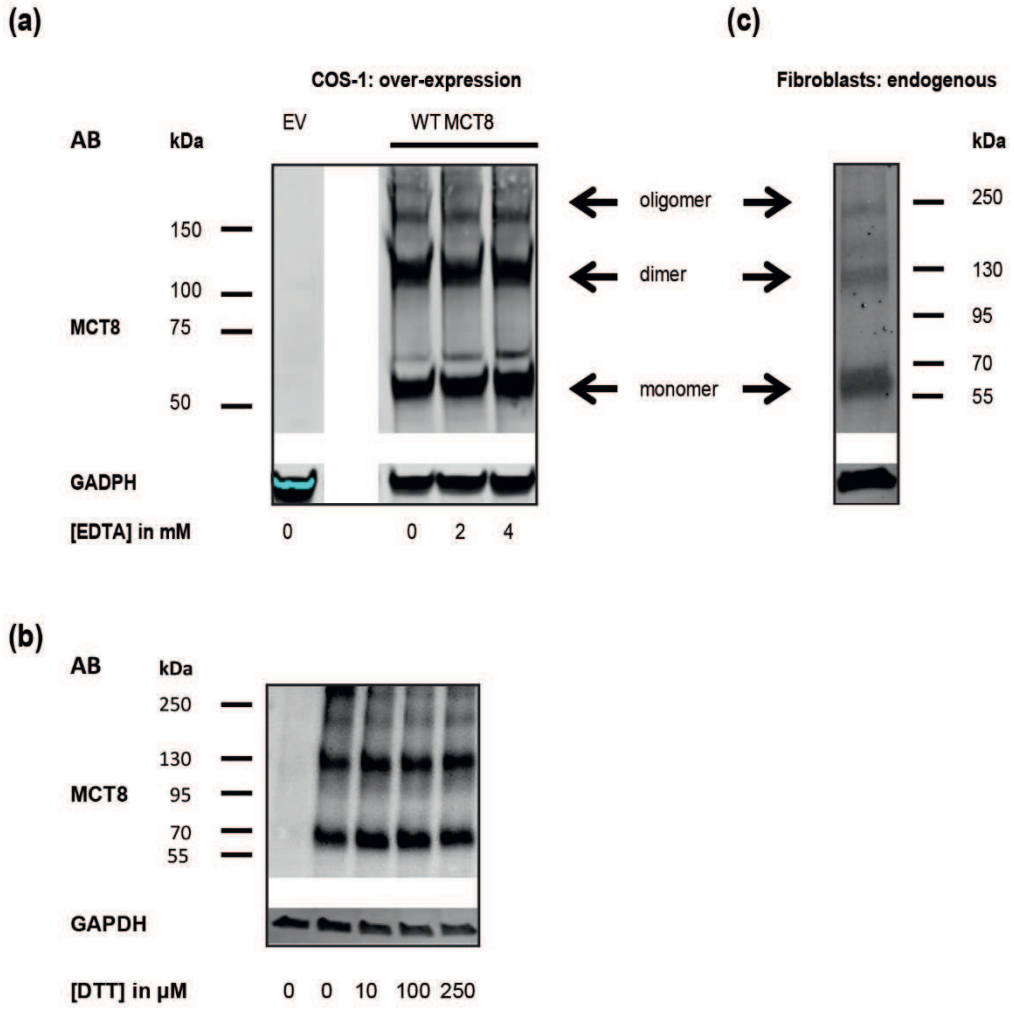
cDNA sequence of coding region of the hMCT8 construct that was used for the generation of all described mutant and tagged MCT8 constructs. This construct only contains the coding sequence of the short MCT8 isoform starting at Met75. The sequence encoding the unique part of the long MCT8 isoform is depicted 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.

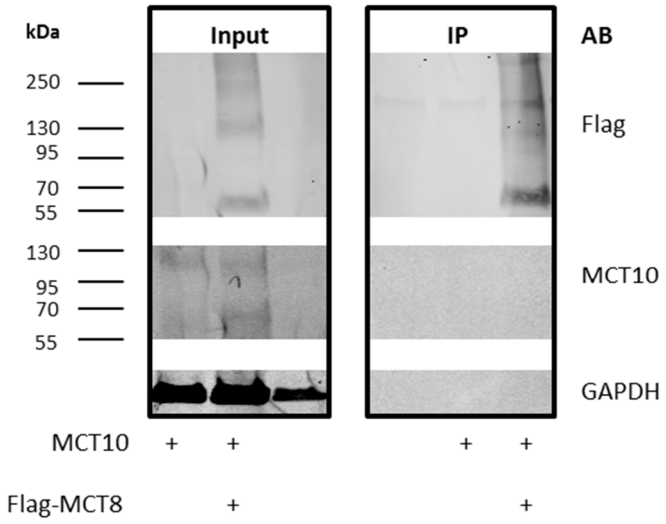
Supplemental Figure 2



(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

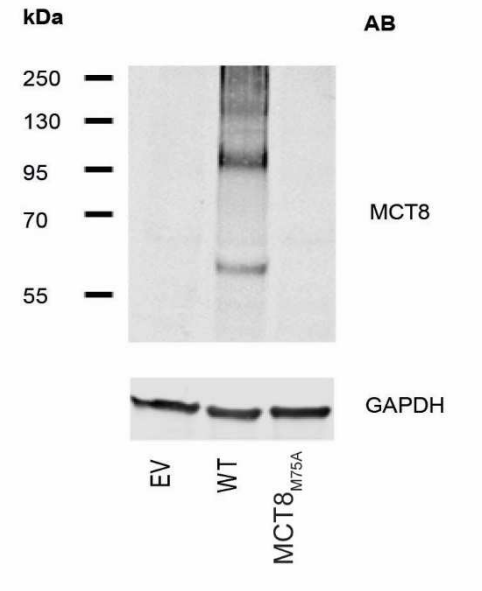
detected using the N-terminal MCT8 antibody (3353) and GAPDH was used as a loading control. **(C)** Immublot on total lysates derived from skin fibroblasts obtained from a healthy subject, using the N-terminal MCT8 antibody (3353) and GAPDH as a loading control. Note that endogenously expressed MCT8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

Supplemental Figure 3



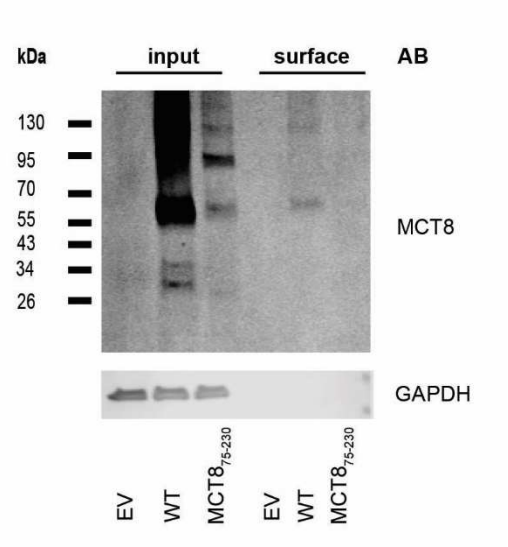
Representative blots (N=3) of co-IP studies on lysates derived from COS-1 cells transiently expressing MCT10 alone (lane 2), or in combination with Flag-MCT8 (lane3). Blots were simultaneously stained with mouse M2 anti-Flag and rabbit anti-MCT10 antiserum (designated 1758). MCT10 could not be enriched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

Supplemental Figure 4



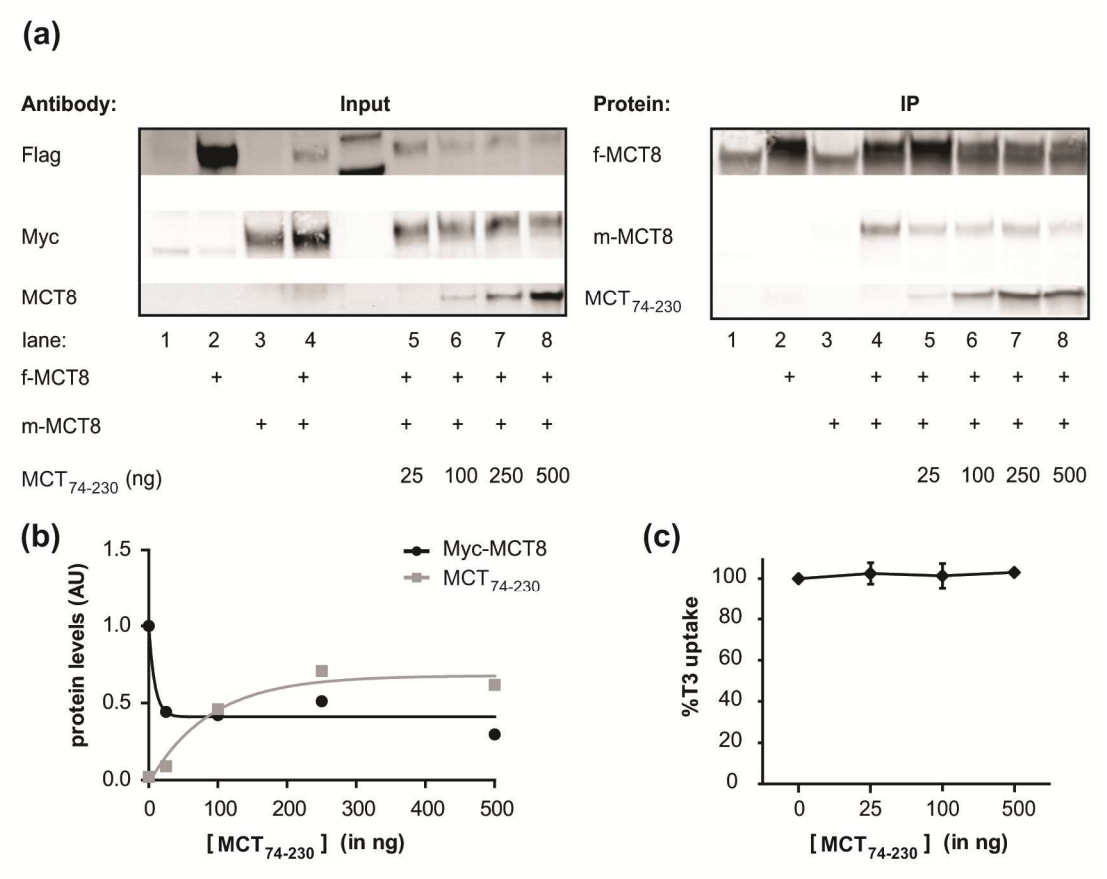
Representative immunoblot of lysates from COS-1 cells transfected with WT MCT8 or the $MCT8_{M75A}$ mutant (N=2), performed as described in Fig. 2D. Note, that no signal was obtained upon inactivation of the natural translation start site (Met75) using antibodies directed against the N-terminus of MCT8. 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
hMCT8	AA 527–539 C-terminus	MCT8 (1306)	Rabbit (p)	Personal AB (19)	1:1000	1:500	AB_2661880
hMCT10	AA 473–487 and 503–515	MCT10 (1758)	Rabbit (p)	Personal AB (19)	1:1000		AB_2661879
GAPDH		GAPDH	Mouse (m)	Millipore (Mab 374)	1:20000		AB_2107445
ZO1		ZO1	Mouse (m)	Thermo Fisher (33-9100)	1:1000	1:1000	AB_2533147
Flag	Flag epitope	Flag-M2	Mouse (m)	Sigma (C3956)	1:1000		AB_439680
Myc	Myc epitope	Myc	Rabbit (m)	Sigma (F1804)	1:1000		AB_262044
Mouse IgG		IRDye680	Goat	LI-COR (926-68020)	1:20000		AB_10706161
Rabbit IgG		IRDye800	Goat	LI-COR (926-32211)	1:20000		AB_621843
Rabbit IgG		Alexa 488	Goat	Thermo Fisher (A11008)		1:1000	AB_143165
Mouse IgG		Alexa 633	Goat	Thermo Fisher (A21050)		1:1000	AB_2535718

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.	