STUDIES ON THE DIDS BINDING SITE OF MONOCARBOXYLATE TRANSPORTER 1 SUGGEST A HOMOLOGY MODEL OF THE OPEN CONFORMATION AND A PLAUSIBLE TRANSLOCATION CYCLE

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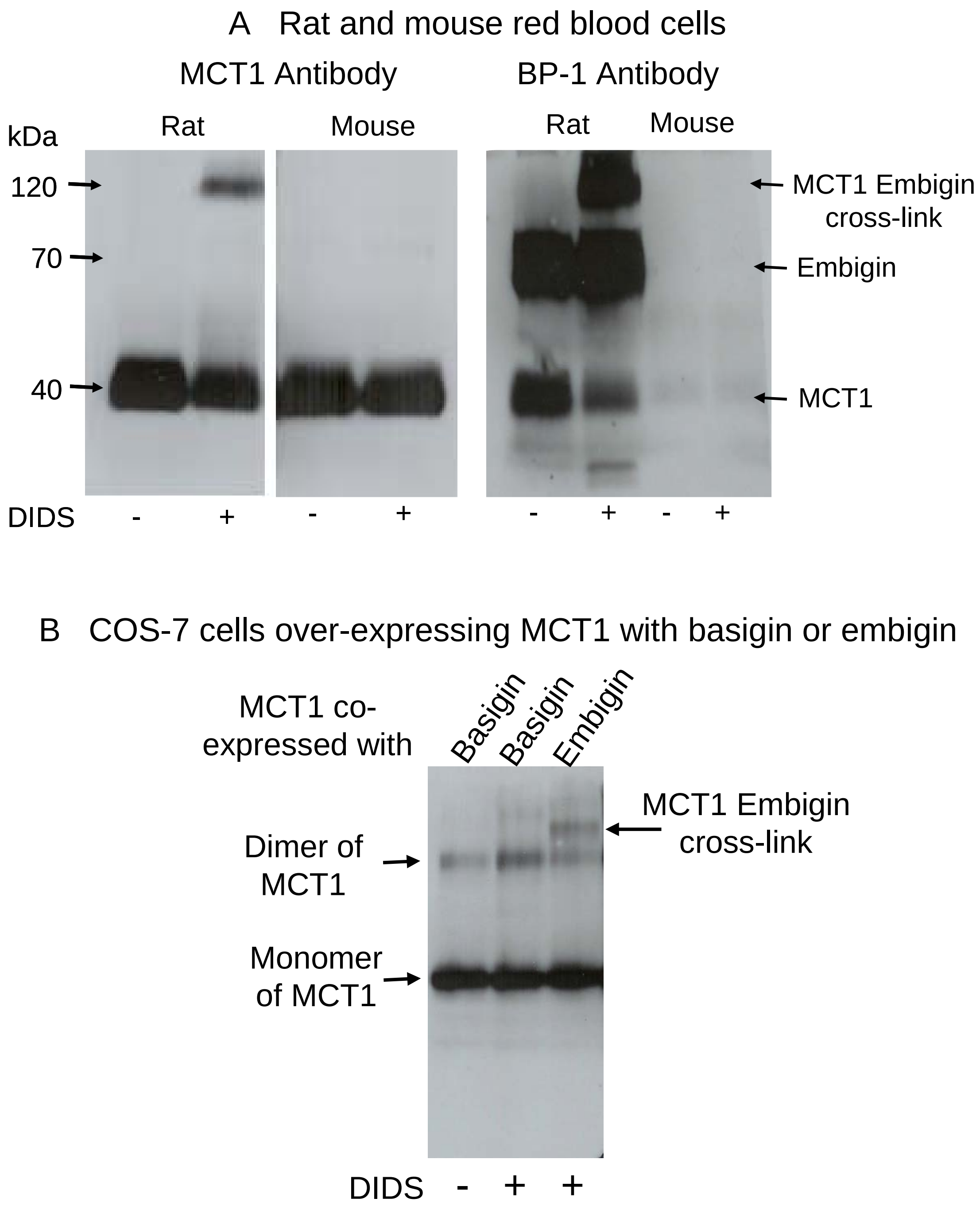
Supplementary Material

Table 1S Primers used in PCR generation of site-directed mutants of MCT1 and embigin

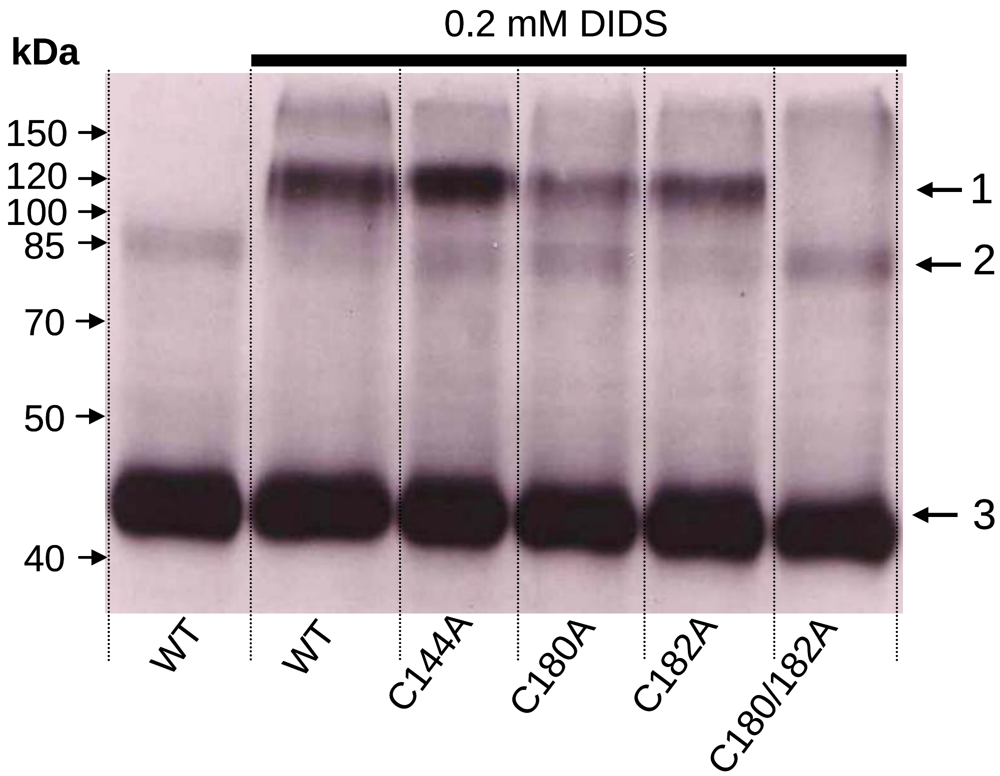
Mutant	Sense Primer	Anti-sense Primer
Rat MCT1 K38Q	cctatgcatttccccaatccatcactgtc	gacagtgatggattggggaaatgcatagg
Rat MCT1 K38R	cctatgcatttcccagatccatcactgtc	gacagtgatggatctgggaaatgcatagg
Rat MCT1 K45Q	ccatcactgtcttctttcaagagattgaaattatat	atataatttcaatctctcttaaagaagacagtgatgg
Rat MCT1 K45R	ccatcactgtcttctttagagagattgaaattatat	atataatttcaatctctctaaagaagacagtgatgg
Rat MCT1 K282Q	ctttcttagtaattatggtcagagtaagcatttttccag	ctggaaaaatgcttactctgaccataattactaagaaag
Rat MCT1 K282R	ctttcttagtaattatggtaggagtaagcatttttccag	ctggaaaaatgcttactcctaccataattactaagaaag
Rat MCT1 K284Q	gtaattatggtaagagtcagcatttttccagtgag	ctcactggaaaaatgctgactcttaccataattac
Rat MCT1 K282Q K284Q	gtaattatggtcagagtcagcatttttccagtgag	ctcactggaaaaatgctgactctgaccataattac
Rat MCT1 K282R K284R	gtaattatggtaggagtaggcatttttccagtgag	ctcactggaaaaatgcctactcctaccataattac
Rat MCT1 K290Q	catttttccagtgagcagtcagccttcctcc	ggaggaaggctgactgctcactggaaaaatg
Rat MCT1 K290R	catttttccagtgagcggtcagccttcctcc	ggaggaaggctgaccgctcactggaaaaatg
Rat MCT1 K413Q	gtatggagactaccaatacacatactggg	cccagtatgtgtattggtagtctccatac
Rat MCT1 K413R	gtatggagactacagatacacatactggg	cccagtatgtgtatctgtagtctccatac
Rat Embigin K104Q K105Q	gaatgtgacttggcagcaagatgacgcgc	gcgcgtcatcttgctgccaagtcacattc
Rat Embigin K120Q	ggtttcaatacaactcaaatgggggacac	gtgtcccccatttgagttgtattgaaacc
Rat Embigin K137Q K141Q	ccgtttttaatagccaacaaatgggacaatactcttgtttcc	ggaaacaagagtattgtcccatttgttggctattaaaaacgg
Rat Embigin K224Q K226Q	cgaaacaaagctccaggtacagcaccttttggagg	cctccaaaaggtgctgtacctggagctttgtttcg
Rat Embigin K252Q	gaggaacacattcagctggttgtgctg	cagcacaaccagctgaatgtgttcctc
Rat Embigin K263Q	catggtgcctctccagccattccttgcc	ggcaaggaatggctggagaggcaccatg
Rat Embigin C144A	gggaaaatactctgctttccttggagaag	cttctccaaggaaagcagagtattttccc
Rat Embigin C180A	ctactgtgctaaaggctgaatgtcaaaattgtc	gacaattttgacattcagcctttagcacagtag
Rat Embigin C182A	gtgctaaagtgtgaagctcaaaattgtcttcc	ggaagacaattttgagcttcacactttagcac
Rat Embigin C180A C182A	ctactgtgctaaaggctgaagctcaaaattgtcttcc	ggaagacaattttgagcttcagcctttagcacagtag

Table 2S Uninhibited rates of lactate transport into *Xenopus laevis* oocytes mediated by the different MCT1 mutants

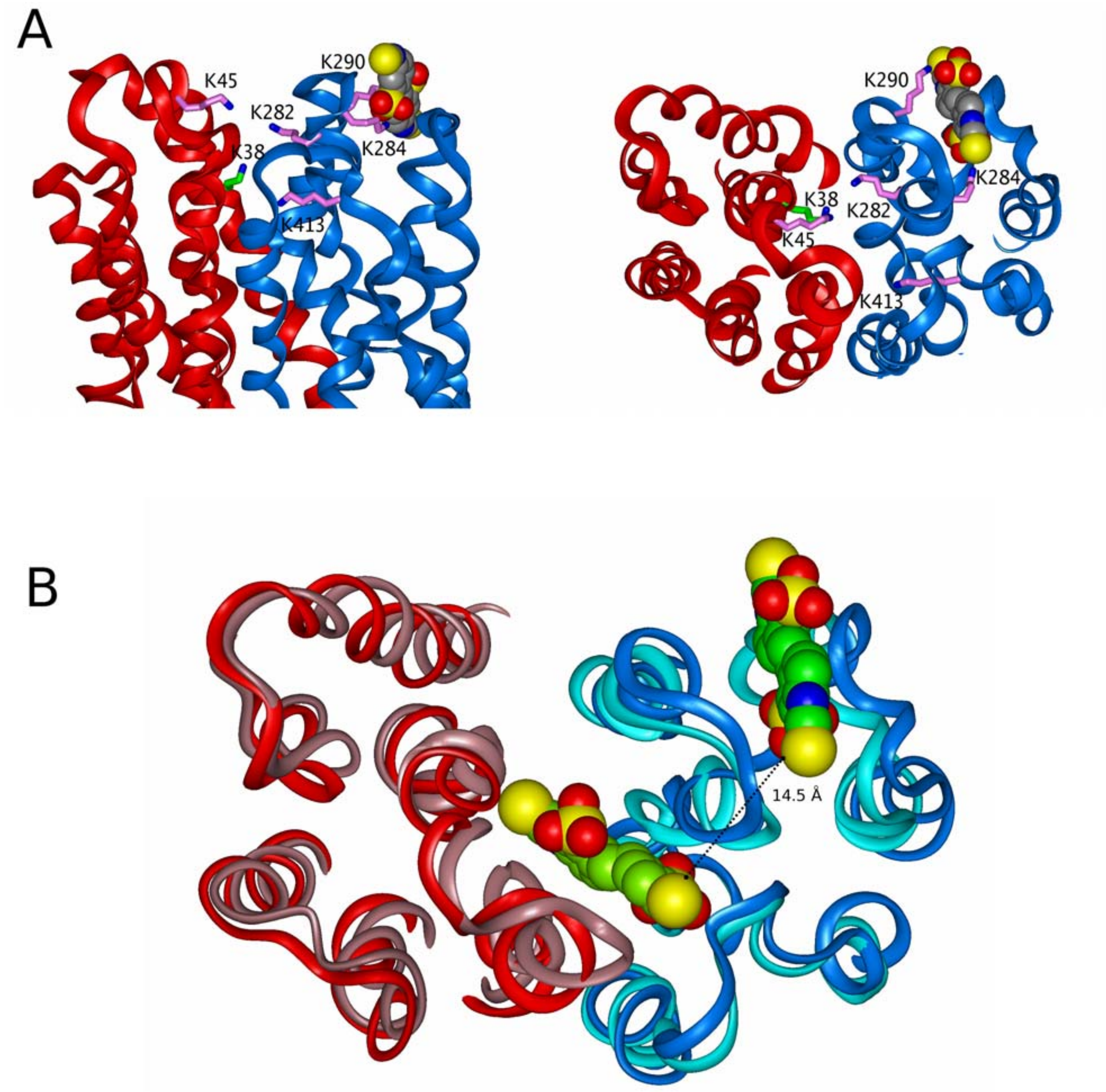
Mutation	Rate of lactate uptake (pMol per egg in 10 min)	N value
Wild type	535 ± 14	167
K38Q	48 ± 7	40
K38R	34 ± 4	30
K45R	500 ± 28	25
K45Q	425 ± 36	25
K282Q	302 ± 38	20
K282R	369 ± 30	26
K284Q	650 ± 46	20
K290Q	390 ± 38	20
K413Q	385 ± 45	17
K413R	446 ± 25	26
K45Q/K282Q	301 ± 15	26
K45R/K282Q	344 ± 17	20
K45Q/K282R	509 ± 23	20
K45R/K282R	656 ± 21	12
K45Q/K413Q	413 ± 19	25
K45Q/K413R	243 ± 18	20
K45R/K413Q	442 ± 38	15
K45R/K413R	485 ± 34	12
K282Q/K284Q	573 ± 43	17
K282R/K284Q	459 ± 27	20
K282Q/413Q	445 ± 25	46
K282Q/K413R	464 ± 41	13
K282R/K413Q	470 ± 25	41
K282R/K413R	426 ± 12	20
K284Q/K413Q	666 ± 62	16
K45/K282Q/K413Q	286 ± 20	24
K45/K284Q/K413Q	420 ± 36	30
K45Q/K282R/K413R	299 ± 27	28
K45R/K282Q/K413Q	405 ± 34	20
K45R/K282Q/K413R	322 ± 21	26
K45R/K282R/K413Q	394 ± 22	20
K45Q/K282R/K413Q	223 ± 14	20
K45R/K282R/K413R	308 ± 12	20
K282Q/K284Q/K413Q	484 ± 13	30
K45Q/K282Q/K413R	239 ± 13	20
K282R/K284Q/K413Q	420 ± 12	20
K45Q/K282Q/K284Q/K413Q	379 ± 31	19
Uninjected	34 ± 1	161
Water	32 ± 1	96



Supplementary Figure 1. DIDS does not cross-link basigin to MCT1. In Panel A rat or mouse erythrocytes were incubated with or without 0.1 mM DIDS and red cell ghost membrane prepared as described in the Experimental section. Samples were separated by SDS-PAGE and Western blots performed using either the C-terminal MCT1 antibody or an antibody to the embigin/MCT1 DIDS cross-linked product (BP-1 antibody) as indicated. In Panel B COS-7 cells were transfected with MCT1 + basigin (Lanes 1 and 2) or MCT1 + embigin (Lane 3). COS cells were harvested 48 hr after transfection and treated ± 0.2 mM DIDS as indicated, washed and lysed. SDS-PAGE and Western blots were performed on the crude lysates using anti-MCT1 antibody.



Supplementary Figure 2 C180A/C182A embigin does not exhibit DIDS cross-linking to MCT1. C180 usually forms the cysteine bridge that stabilises the V immunoglobulin domain of embigin, although our data suggest that C182 may also fulfil this role in the absence of C180. Preventing this cross-link is thought to disrupt this V domain and prevent correct alignment of K160 and K164 of DIDS to allow cross-linking to MCT1.



Supplementary Figure 3 An alternative weak binding site for DIDS on MCT1

In Panel A presents sideways (left) and top-down (right) views of an energy-minimised model that shows DIDS binding to a shallow groove between the loops connecting helices 7 & 8 and helices 9 & 10. K284 forms a salt bridge with one sulfonate group of DIDS while the tip of K290 is in Van de Waals contact with the isothiocyanate group at the other ring of DIDS. Colouring and views are analogous to those of Figures 7 and 9 in main paper. In Panel B this weak binding site (darker shade) is overlaid on the tight binding site (lighter shade) to show the displacement of the emerging isothiocyanate group involved in cross-linking to embigin.