

Supplementary Figure 1. SLC35A transporters mRNA expression in skeletal muscle and brain

mRNA level of expression of the SLC35A1-A2-A3-A4-A5 transporter in skeletal muscle and some parts of the brain. Data were gathered from Genotype-Tissue Expression (GTEx) Portal. mRNA levels are log10 of transcripts per million.

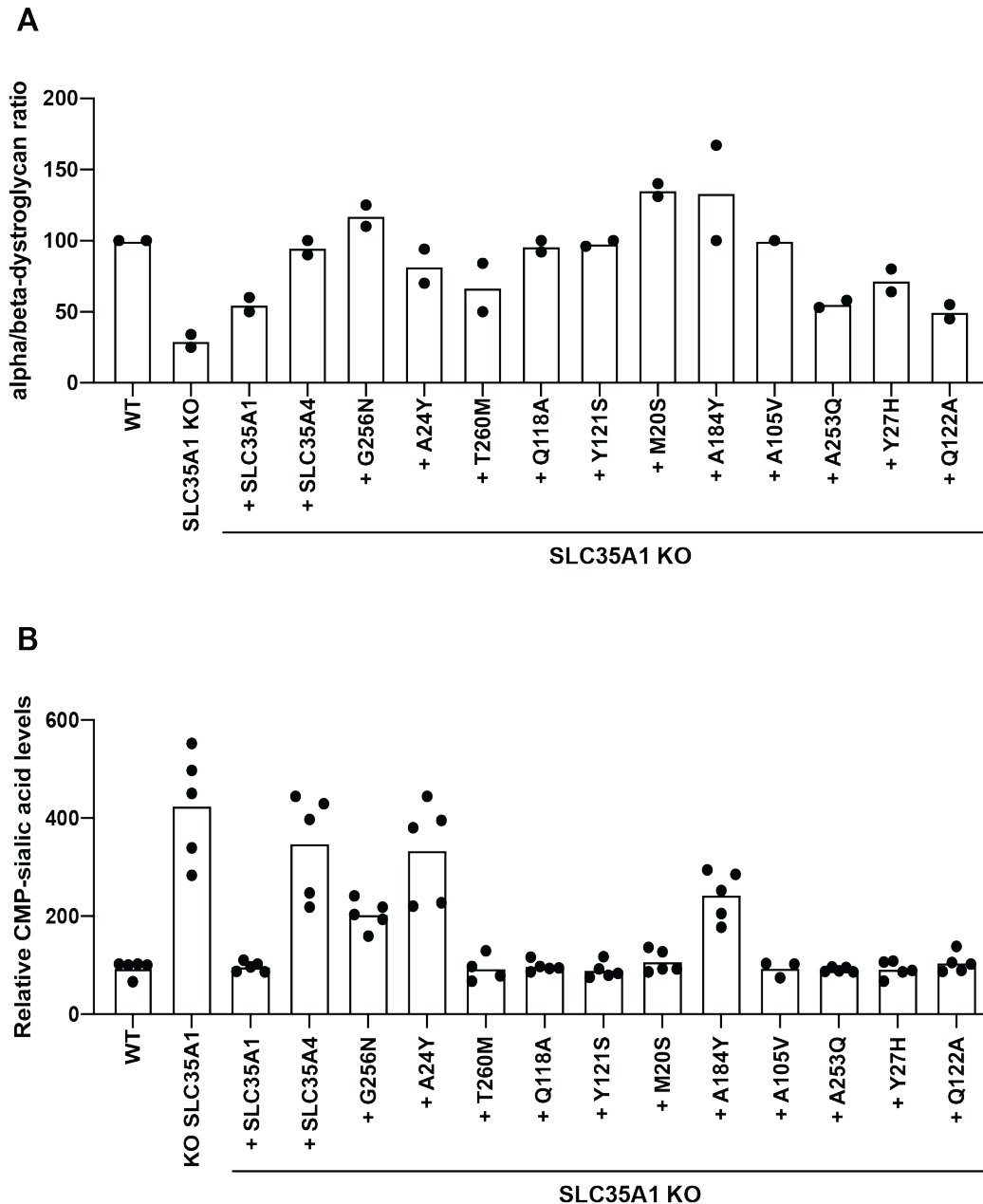
mA1	hA2	hA3	hA4	hA5	moiety interaction
T 51	A 74	A 46	T 57	S 58	none
E 52	E 75	E 47	E 58	E 59	none
Q 118	Q 142	Q 114	Q 116	V 124	none
Q 122	Q 146	Q 118	N 120	N 128	none
T 128	T 152	T 124	T 126	T 134	none
Q 159	Q 183	Q 155	A 157	E 208	none
A 184	S 209	A 182	Y 195	Q 239	none
G 189	G 214	G 187	G 200	S 244	none
G 192	G 217	G 190	S 203	N 247	none
R 209	R 234	R 207	Q 220	Q 267	none
V 264	V 289	I 262	M 271	L 324	none
G 273	G 298	G 271	L 280	V 333	none
A 276	T 301	T 274	V 283	A 336	none
I 280	I 305	I 278	L 287	T 340	none
K 55	K 78	K 50	K 61	K 62	CMP
Y 98	Y 122	Y 94	Y 96	Y 104	CMP
S 188	S 213	S 186	S 199	S 243	CMP
F 195	F 220	F 193	T 206	N 250	CMP
N 210	N 235	N 208	N 221	N 268	CMP
M 213	L 238	L 211	L 224	L 271	CMP
Y 214	G 239	G 212	Y 225	Y 272	CMP
G 257	G 282	G 255	G 264	G 317	CMP
S 261	A 286	A 259	S 268	A 321	CMP
Q 101	Q 125	Q 97	N 99	D 107	CMP & Sia
N 102	N 126	N 98	N 100	N 108	CMP & Sia
Y 121	Y 145	Y 117	S 119	S 127	CMP & Sia
K 124	K 148	K 120	K 122	S 130	CMP & Sia
T 260	V 285	I 258	M 267	V 320	CMP & Sia
K 272	K 297	K 270	R 279	H 332	CMP & Sia
M 20	L 43	L 13	S 26	F 26	Sia
V 23	Q 46	Q 16	M 29	L 29	Sia
A 24	N 47	T 17	Y 30	S 30	Sia
Y 27	L 50	L 20	H 33	R 33	Sia
T 28	I 51	V 21	A 34	I 34	Sia
A 105	Q 129	L 101	V 103	V 111	Sia
Y 117	F 141	Y 113	Y 115	A 123	Sia
A 253	Q 278	Q 251	Q 260	T 313	Sia
G 256	G 281	G 254	N 263	Q 316	Sia

Color Code:

- conserved among all sequences
- conserved between mA1 and two other sequences
- conserved between mA1 and three other sequences
- conserved between mA1 and one other sequence
- conserved between hA2 and hA3 only
- conserved between hA4 and hA5 only
- residue is conserved between hA5 and both hA2 and hA3
- residue is conserved between hA4 and both hA2 and hA3
- residue is conserved between hA4, hA2, hA3 and hA5

Supplementary Figure 2. Comparison of cavity-lining residues among SLC35A family members.

In the left-most column, residues that line the internal substrate-binding cavity in the structure of mouse CST (mSLC35A1) in complex with CMP-Sia are listed along with their position in the sequence. In the next four columns, the corresponding residues are listed for the human sequences of SLC35A2, SLC35A3, SLC35A4 and SLC35A5. The last column indicates which part of CMP-Sia interacts with the CST residues listed in the first column.



Supplementary figure 3. Effect of the expression of mutant SLC35A1 proteins on CMP-sialic acid levels and laminin binding in SLC35A1 knockout cells.

(A) The intensity of the laminin overlay band relative to the band of the β -dystroglycan western blot was assessed in wild type and SLC35A1 knockout HAP1 cells, where the indicated mutants or wild type SLC35A1 and SLC35A4 were expressed. Values are means of two experiments.

(B) Relative CMP-sialic acid levels were determined by LC-MS in the cell lines described in A. Values were normalized within each experiment to levels in wild-type (control) cells and are means of two experiments performed in duplicates and triplicates, respectively.

Supplementary table 1. Primers used in this work

List of PCR primers used in the course of this work to perform all the necessary cloning.

mSlc35a1 Open Reading Frame PCR primers	
mSlc35a1-NheI_Sense	5'-ATA CAA GCT AGC CAG ACT CTC TCG GCA CCA TG – 3'
mSlc35a1-EcoRI_Reverse	5'- CAC ACC GAA TTC TCA CTT GTC GTC ATC GTC TTT GTA GTC CAC ACC AAT GAT TCT CTC TTT TGA AG-3'
hSLC35A4 Open Reading Frame PCR primers	
hSLC35A4-BbsI_Sense	5'-ATA CAT AGA AGA CAT CTA GCC AGG GTC TTG TGT GGG T- 3'
hSLC35A4-BbsI_Reverse	5'- TAT AAG GAA GAC AAA ATT TCA CTT GTC GTC ATC GTC TTT GTA GTC GCG GCT GCC ATA GTA CAG GC-3'
shRNA amplification primers	
miR-E-XhoI_Sense	5'- AGA AGG CTC GAG AAG GTA TAT TGC TGT TGA CAG TGA GCG -3'
miR-E-EcoRI_Reverse	5'- GCT CGA ATT CTA GCC CCT TGA AGT CCG AGG CAG TAG-3'
SLC35A1 mutagenesis primers	
mSlc35a1-G256N_Sense	5'- CTT CCT TGC TAG TGT GAA CGG CCT CTA CAC GTC AG – 3'
mSlc35a1-G256N_Reverse	5'- CTG ACG TGT AGA GGC CGT TCA CAC TAG CAA GGA AG – 3'
mSlc35a1-A24Y_Sense	5'- GTG ATG ACT CTG GTG TAT GCC GCT TAC ACC G – 3'
mSlc35a1-A24Y_Reverse	5'- CGG TGT AAG CGG CAT ACA CCA GAG TCA TCA C – 3'
mSlc35a1-A184Y_Sense	5'- GCT TTG GTG CTA TAG CTA TTT ACG TAT TGT GCT CTG GAT TTG C – 3'
mSlc35a1-A184Y_Reverse	5'- GCA AAT CCA GAG CAC AAT ACG TAA ATA GCT ATA GCA CCA AAG C – 3'
mSlc35a1-Y121S_Sense	5'- GTA CCA GGT GAC CTC TCA ACT GAA GAT CC – 3'
mSlc35a1-Y121S_Reverse	5'- GGA TCT TCA GTT GAG AGG TCA CCT GGT AC– 3'
mSlc35a1-T260M_Sense	5'- GAG GCC TCT ACA TGT CAG TGG TGG TG – 3'
mSlc35a1-T260M_Reverse	5'- CAC CAC CAC TGA CAT GTA GAG GCC TC – 3'
mSlc35a1-M20S_Sense	5'- GCT TGG CGG TGA GTA CTC TGG TGG CTG – 3'
mSlc35a1-M20S_Reverse	5'- CAG CCA CCA GAG TAC TCA CCG CCA AGC – 3'
mSlc35a1-A253Q_Sense	5'- GGT TTG TTA TCT TCC TTC AAA GTG TGG GAG GCC TCT AC – 3'
mSlc35a1-A253Q_Reverse	5'- GTA GAG GCC TCC CAC ACT TTG AAG GAA GAT AAC AAA CC – 3'
mSlc35a1-A105V_Sense	5'- GCA GAA CAA CAT GGT CTT CCT GGC TCT C – 3'

mSlc35a1-A105V_Reverse	5'- GAG AGC CAG GAA GAC CAT GTT GTT CTG C – 3'
mSlc35a1-Y27H_Sense	5'- CTG GTG GCT GCC GCT CAC ACC GTA GCT TTA AG – 3'
mSlc35a1-Y27H_Reverse	5'- CTT AAA GCT ACG GTG TGA GCG GCA GCC ACC AG – 3'
mSlc35a1-Q118A_Sense	5'- GAT GCA GCA GTG TAC GCG GTG ACC TAT CAA C – 3'
mSlc35a1-Q118A_Reverse	5'- GTT GAT AGG TCA CCG CGT ACA CTG CTG CAT C – 3'
mSlc35a1-Q122A_Sense	5'- GTA CCA GGT GAC CTA TGC ACT GAA GAT CCC CTG – 3'
mSlc35a1-Q122A_Reverse	5'- CAG GGG ATC TTC AGT GCA TAG GTC ACC TGG TAC – 3'
Hifi_UB82-EcoRI-XbaI-Sense	5'- TAG GCT TTT GCG GAT CCT TGG GTT T – 3'
Hifi_UB82-EcoRI-XbaI-Reverse	5'- GAA AAG CGC CTC CCC TAC CCG GTA G – 3'
SLC35A1 and SLC35A4 shRNA sequence	Plasmid
SLC35A1 – shRNA 1 - Sense	5'-TGC TGT TGA CAG TGA GCG AGA ATT GTA TTA TTG TGT GTA ATA GTG AAG CCA CAG ATG TA-3'
SLC35A1 – shRNA 1 - Reverse	5'-TGA AGT CCG AGG CAG TAG GCA GGA ATT GTA TTA TTG TGT GTA ATA CAT CTG TGG CTT CAC-3'
SLC35A1 – shRNA 2 - Sense	5'-TGC TGT TGA CAG TGA GCG CGC AGT CTA TAC CAT AGC TTT ATA GTG AAG CCA CAG ATG TA-3'
SLC35A1 – shRNA 2 - Reverse	5'-TGA AGT CCG AGG CAG TAG GCA TGC AGT CTA TAC CAT AGC TTT ATA CAT CTG TGG CTT CAC-3'
SLC35A1 – shRNA 3 - Sense	5'-TGC TGT TGA CAG TGA GCG CAC AAG GAG AAA CAG CTT CAA ATA GTG AAG CCA CAG ATG TA-3'
SLC35A1 – shRNA 3 - Reverse	5'-TGA AGT CCG AGG CAG TAG GCA AAC AAG GAG AAA CAG CTT CAA ATA CAT CTG TGG CTT CAC-3'
SLC35A4 – shRNA 1 - Sense	5'-TGC TGT TGA CAG TGA GCG ATG ACC TCT CTG TTC ACC CTG ATA GTG AAG CCA CAG ATG TA-3'
SLC35A4 – shRNA 1 - Reverse	5'-TGA AGT CCG AGG CAG TAG GCA CTG ACC TCT CTG TTC ACC CTG ATA CAT CTG TGG CTT CAC-3'
SLC35A4 – shRNA 2 - Sense	5'-TGC TGT TGA CAG TGA GCG CG GTG ATC TAT CTT CAG CGT TAT AGT GAA GCC ACA GAT GTA-3'
SLC35A4 – shRNA 2 - Reverse	5'-TGA AGT CCG AGG CAG TAG GCA TGG TGA TCT ATC TTC AGC GTT ATA CAT CTG TGG CTT CAC-3'
SLC35A4 – shRNA 3 - Sense	5'-TGC TGT TGA CAG TGA GCG CGC CAC AGA TTA TAC AAC CAT ATA GTG AAG CCA CAG ATG TA-3'
SLC35A4 – shRNA 3 - Reverse	5'-TGA AGT CCG AGG CAG TAG GCA TGC CAC AGA TTA TAC AAC CAT ATA CAT CTG TGG CTT CAC-3'

Supplementary table 2. Targeted sequence for CRISPR/Cas9 Knock-out

List of sequenced targeted by CRISPR/Cas9 for the preparation of cells knocked-out for ISPD, SLC35A1 and SLC35A4. Codon written in lower case represents the PAM sequence.

Targeted Gene	Targeted Sequence
ISPD – 1	GTCATCAGCTACACCCTAC <u>agg</u>
ISPD – 2	<u>ccc</u> ACCCCGAAGCAATTCTGCCC
SLC35A1 – 1	CTGGAGTTACGCTTGTACAG <u>tgg</u>
SLC35A1 – 2	<u>ccg</u> GACACTCAGCAAATTACAGT
SLC35A4 – 1	GGCACCGCCTCTCTGTGCGT <u>Cagg</u>
SLC35A4 – 2	<u>cct</u> GCATATCACTCCGCTAGGCC