

Supplementary information

Structural insights into inhibitory mechanism of human excitatory amino acid transporter EAAT2

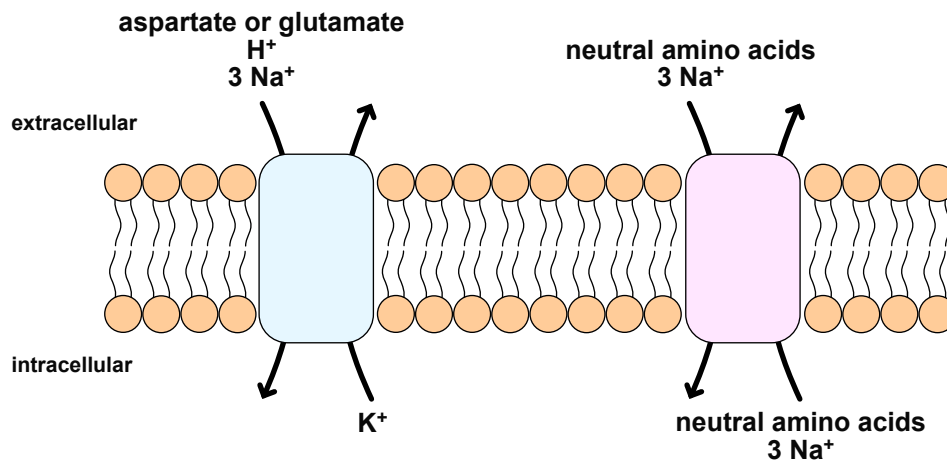
Kato et al.,

Supplementary Figures 1-10

Supplementary Table 1

EAAT1-5

ASCT1, 2



Supplementary Figure 1 | Substrates and coupling ions of SLC1A transporters

Amino acid transport by all SLC1As is coupled to three extracellular sodium ions. In addition to sodium ions, extracellular proton and intracellular potassium ions are utilized for the transport cycle of EAATs.

1 10 20 30 40

EAAT2 MASTEGANNMPKQVEVRMHDShLGSEEPKHRHLGLRLCDKLGK^NLL^T

EAAT1 MTKSNGEEPKMGRMERFQQGVRRKRTLLAKKKVQNITKEDVKSylFR^NAV^L

EAAT3 MG..... KPARKGCEWKRFLKNN^NWVL^L

EAAT4 MSSHGNSLFLRESGQRLGRVGLWLRQLQESLQQRALRTRLRLQTMTLEHVLRFLRR^NAF^IL

EAAT5 MVPHAILARGR..... DVCRR^NGLLI^I

ASCT1 MEKSNETNGYLDsAQAG..... PAAGPGAPGTAAG..... RARRCAGFLRR^NQALVL^L

ASCT2 MVADPPRDSKGLAAAEPTANGGLALASIEDQGAAGGYCGSRDQVRRCLRR^NLLVL^L

Gltph MG...LYRKYIEY^NPVL^LQ

Glttk MGKSLRRYLDY^NPVL^LW



50 60 70 80 90 100

EAAT2 LTVFGVILGAVCGGLRLASP...THPDVVM^LIAFPGDILMRMLKMLII^FLIIS^NSLIT^TGL

EAAT1 LTVTA^NVIVGTILGFTLRPYR...MSYREVKYFSFPGELLMRMLQMLVI^FLIIS^NSLVT^TGM

EAAT3 STVAAVVLGITTGVLRVREHSN...LSTLEKFYFAFPGELLMRMLKLI^ILIIS^NSMIT^TGV

EAAT4 LTVSAVVI^NGVSLAFALRPYQ...LTYRQIKYFSFPGELLMRMLQMLVI^FLIIS^NSLVT^TGM

EAAT5 LSVLSVIVGCLLGFLLRTRR...LSPQEISYFQFPGELLMRMLKMMII^FLVVS^NSLMS^NGL

ASCT1 LTVSGVLAGLGLGAALRG...LSLSRTQVTYLAFPGEMLMRMLRMII^FLVVCS^NLVSGA

ASCT2 LTVVA^NVAGVALGLGVSAGGALALGPERLSAFVFPGELLRLRLRMII^FLVVCS^NLVSGA

Gltph KILIGLILGAI^NGLILGHYG...YADAVKTYVKPF^NGDLFVRLKMLVM^FIVFAS^NLVVGA

Glttk KILWGLVLGA^NVFGLIAGHFG...YAGAVKTYVKPF^NGDLFVRLKMLVM^FIVL^NAS^NLVVGA



110 120 130 140 150

EAAT2 SGLDAKASGRIGTRAMVYYMS^NTTII^NAVLGVLVLA^NHPGNPKLKKQLG.....PGKKN

EAAT1 AALDSKASGKMGR^NAVVYYMT^NTTII^NAVIGIIIV^NIIHPGKGTENMH.....REGKI

EAAT3 AALDSNVSGKIGLR^NAVVYYFC^NTTII^NAVILGIVLV^NSIKPGVTQKVGEIA.....RTGST

EAAT4 ASLDNKATGRMGMR^NAAVYYMV^NTTII^NAVFIGILMV^NTIHPGKGSKEGLH.....REGRI

EAAT5 ASLDAKTS^NSRLGVLTVAYYLW^NTTFM^NAVIVGIFMV^NSIHPGSAAQKETT.....EQSGK

ASCT1 ASLDASCLGRIGGI^NAVAYFGL^NTTLSASALAVALA^NFIKPGSGAQTLQSSDLGLEDSGPPP

ASCT2 ASLDPGALGRIGAW^NALLFFLV^NTTLSASALGVGLALAL^NQPGSAASAINASVGAAGSAENA

Gltph ASISPARLGRVG^NVKIVVYYLT^NSAPAV^NTLGIIMAR^NLFNPGAGIHLAVGG.....QQ

Glttk ASISPARLGRVG^NVKIVVYYLA^NSAMAV^NFFGLIVGR^NLFNVGANVNLGSGT.....GK



160 170 180 190 200

EAAT2 DEVSLD^NAF^NLDLIRNLF^NENLVQ^NAC^NFFQIQTVTKKVLVAPPDPDEEANATS.....

EAAT1 VRVTAAD^NAF^NLDLIRNMF^NENLVE^NAC^NFFQFKTNYEKRSFKVPIQANETLVG.....

EAAT3 PEVSTVD^NAM^NLDLIRNMF^NENLVQ^NAC^NFFQYK...TKREEVKPPSDPEMNMTE.....

EAAT4 ETIPTAD^NAF^NMDLIRNMF^NENLVE^NAC^NFFQFKTQYSTRV^NTRTMVRTENGSEPGASMPFFFS

EAAT5 PIMSAD^NAL^NLDLIRNMF^NENLVE^NAT^NFFQYRTKTTPVVKSPKVAPEEAPPRIILYIG...

ASCT1 VPKE^NTVD^NSF^NLDLARNLF^NENLVV^NAA^NFR^NTYATDYKVVTQNSSSGNV.....

ASCT2 PQKEVLD^NSF^NLDLARNLF^NENLVV^NAA^NFR^NSYSTTYEERNITG.....

Gltph FQPKQAP^NPL^NVKILLDIV^NETNPF^NGLANGQVLP^NTIFFAI^NILG.....

Glttk AIEAQPPSL^NVQTLLNIV^NETNPF^NGLANGQVLP^NTIFFAI^NILG.....



210 220 230 240 250 260

EAAT2 AVVSLLN^NETVTEVPEETKMVIKGL^NEFDKGMNVLGLIG^NFFIA^NFGI^NAMGKMGD

EAAT1 AVINN^NVSEAMETL^NTRIT...EELVPV...PGSVNGVNALGLVVF^NSMCF^NGFVIGNMKE

EAAT3 ESFTAVMTT^NAI^NSKNKTKEYKIVG...MYSDGINVLGLIV^NFC^NLVFGLVIGKMGE

EAAT4 VENGTSFLENVTRALGTLQ^NEMLSFEETVPV...PGSANGINALGLVVF^NSAFGLVIGMKH

EAAT5 VQEENGSHVQNFALD^NLTPPEVVYKSEPGTSDGMNVLGIV^NFFSATM^NGIMLGRMGD

ASCT1 THEKIPIG...TEIEGMN^NILGLVLF^NALVLGVALKKLGS

ASCT2 TRVKVPVG...QEVEGMN^NILGLVVF^NAI^NVF^NGV^NALRKLGP

Gltph IA^NIT^NYLMNSEN^NEKV^NRK

Glttk IA^NIT^NYLMNSEN^NEKV^NRK



270 280 290 300 310 320

EAAT2 QAKLMVD^NFFNI^NLNEIV^NMK^NLVIMIMW^NSP^NLGIA^NCHICGKI^NIAIK^NDLEVVARQLGM^NYMTVI

EAAT1 QQQA^NLREFFDS^NLNEA^NIMRLVAVIMW^NAPVGI^NLE^NIA^NGKIVEM^NDMGVI^NGGQLAM^NYTVT^NVI

EAAT3 KGQILVD^NFFNALS^NDATMK^NIVQIMC^NMP^NLGIL^NLE^NIA^NGKIEVED^NDWEIFR^NKLGLYMATVL

EAAT4 KGRVL^NRD^NFFDS^NLNEA^NIMRLVGI^NIIW^NAPVGI^NLE^NIA^NGKILEMD^NDMAVLGGQLGM^NYTVT^NVI

EAAT5 SGAPLV^NSFCQCLNESVMK^NIVAVAVW^NYFPFGIV^NLE^NIA^NGKILEMD^NDPRAVGKKLGFYSVTVV

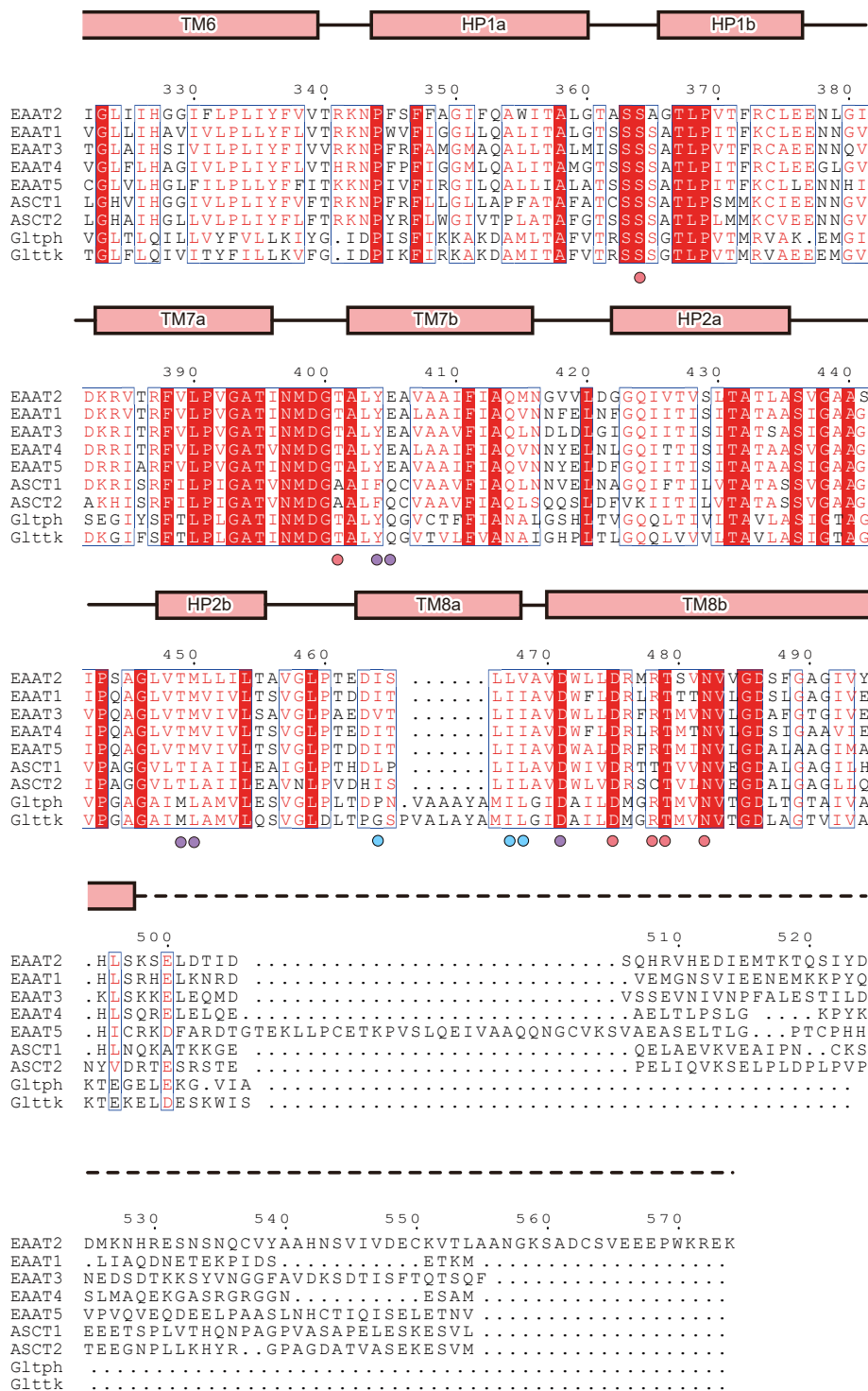
ASCT1 EGEDLIR^NFFNS^NLNEAT^NMVLVSWIMW^NYVPVGI^NLE^NIA^NGKIVEM^NKDII^NVLVTSLGKYIFAS

ASCT2 EGELLIR^NFFNS^NFNEAT^NMVLVSWIMW^NYVPVGI^NLE^NIA^NGKIVEM^NDVGLLFARLGKYILCCL

Gltph SAETLLDAING^NLAEAMYK^NIVNGVMQ^NYAPIGVFA^NLIA^NYVMAEQG...V^NKVVGELAKV^NTA^NAVY

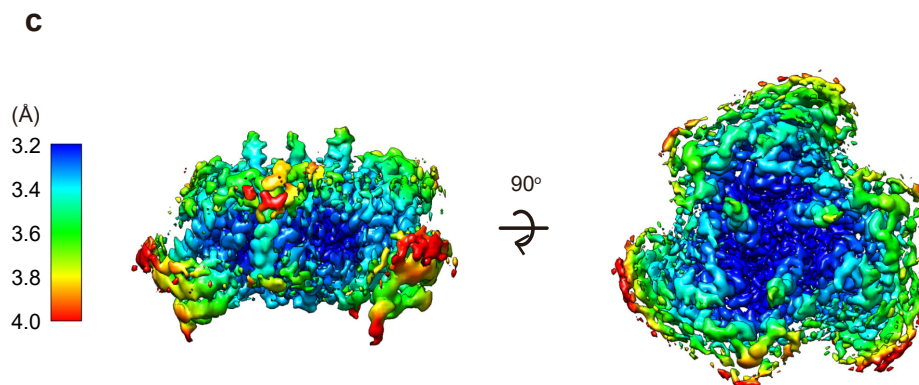
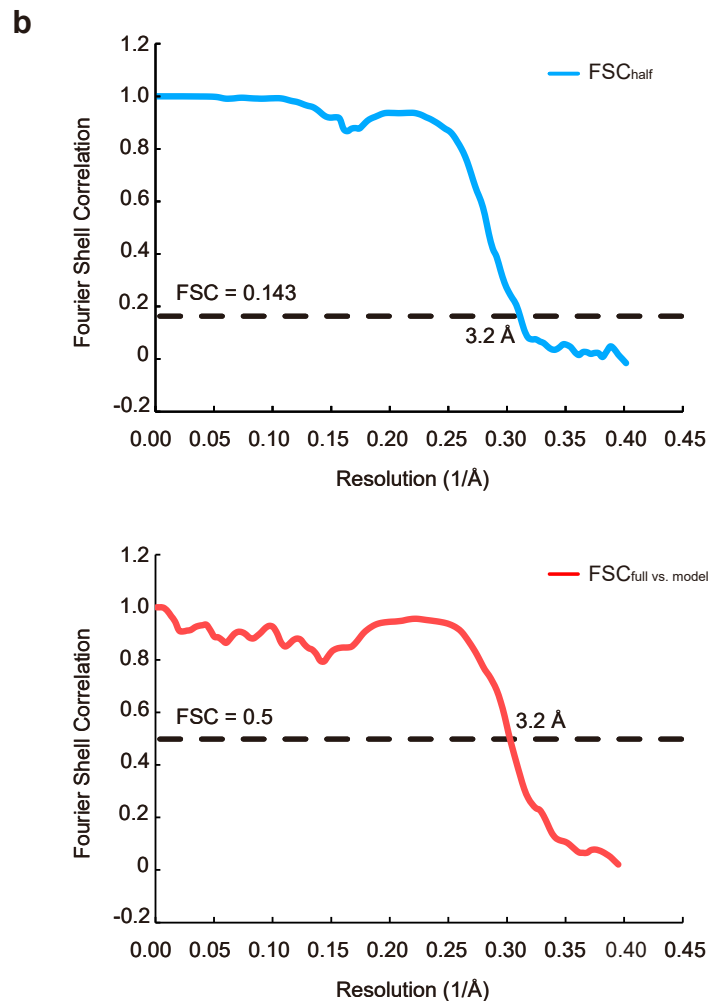
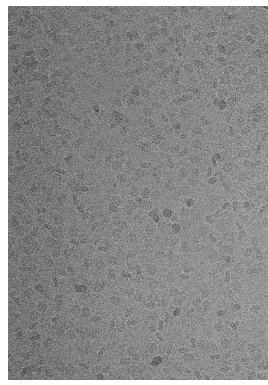
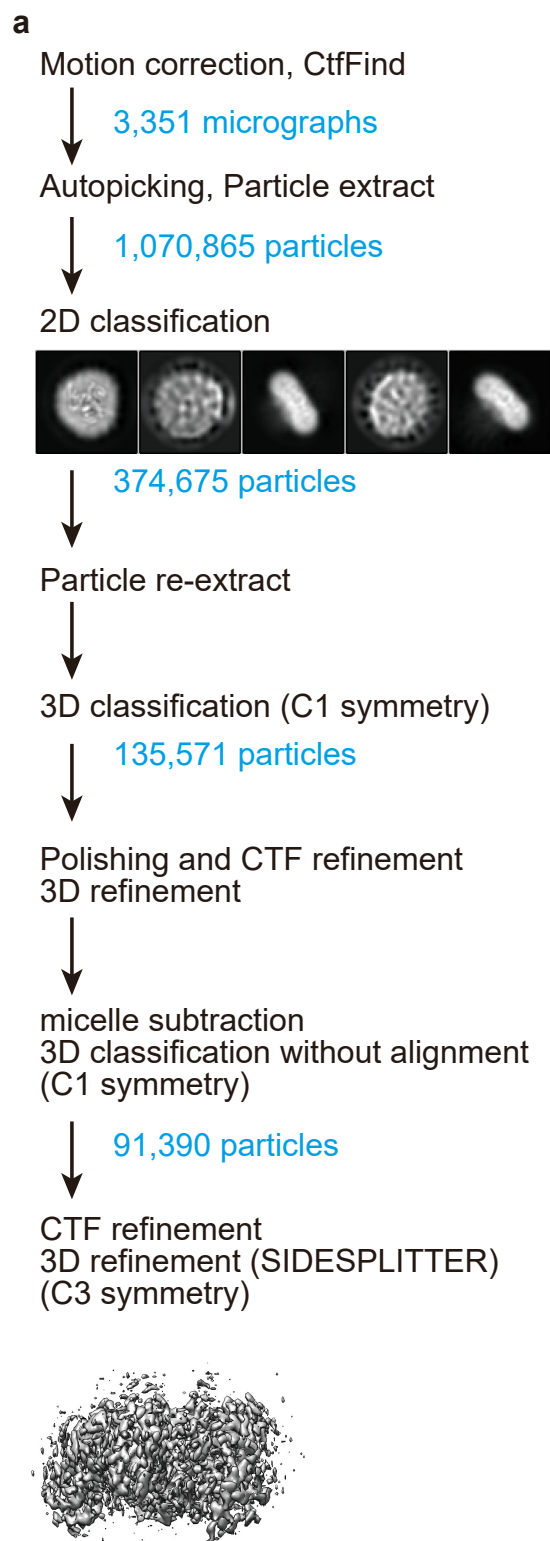
Glttk SAETLLRV^NFDG^NLAEAMYL^NIVGGVMQ^NYAPIGVFA^NLIA^NYVMAEQG...V^NRVVGPLAKV^NTVGAVY





Supplementary Figure 2 | Multiple amino acid sequence alignment of EAAT2

Sequence alignment of *Homo sapiens* EAAT2 (HsEAAT2: UniProt ID P43004), *Homo sapiens* EAAT1 (P43003), *Homo sapiens* EAAT3 (P43005), *Homo sapiens* EAAT4 (P48664), *Homo sapiens* EAAT5 (O00341), *Homo sapiens* ASCT1 (P43007), *Homo sapiens* ASCT2 (Q15758), *Pyrococcus horikoshii* Glt_{ph} (O59090) and *Thermococcus kodakarensis* Glt_{tk} (Q5JID0). The secondary structure of HsEAAT2 is indicated above the sequence. The α -helices, β -strands and disordered regions are indicated by cylinders, arrows and dashed lines, and the scaffold domain and the transport domain are colored light blue and light red, respectively. At the scaffold domain, blue and green triangles indicate kink-inducing residues and Trp286, respectively. At the transport domain, red, purple and blue circles indicate residues of the glutamate-binding site, the cavity the tip around WAY213613, respectively.

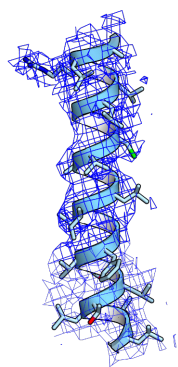


Supplementary Figure 3 | cryo-EM analysis of substrate-free HsEAAT2

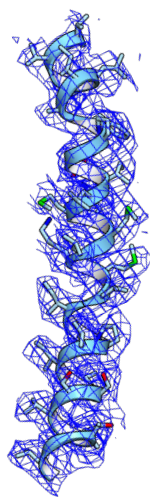
a, Flow chart of cryo-EM data processing of HsEAAT2 substrate-free structure. **b**, Fourier Shell Correlation (FSC) curve of the final 3D reconstruction model calculated using "relion_postprocess" with masked marked 3.2 Å resolution, corresponding to the FSC = 0.143 gold standard cut-off criterion (blue). FSC curve of map vs. model (red). **c**, Local resolution of the HsEAAT2 substrate-free structure, estimated by RELION.

a

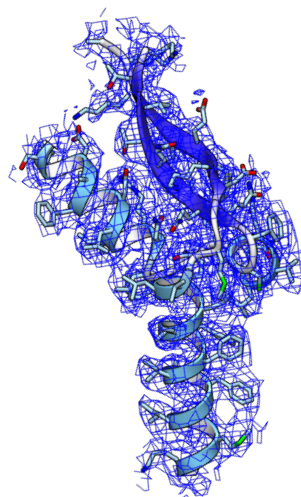
Scaffold domain



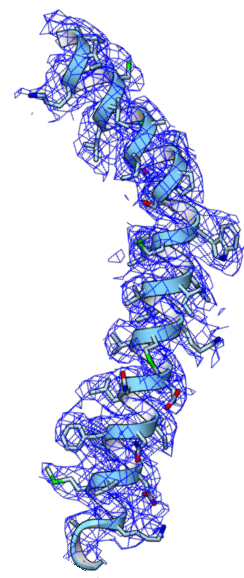
TM1



TM2



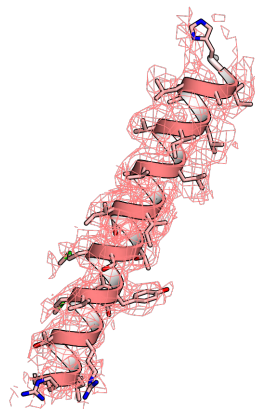
TM4



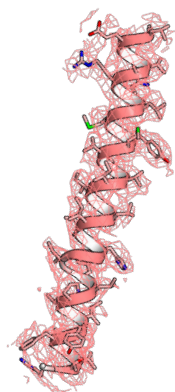
TM5

b

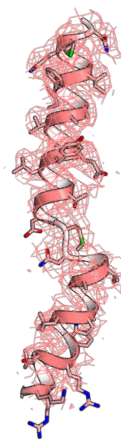
Transport domain



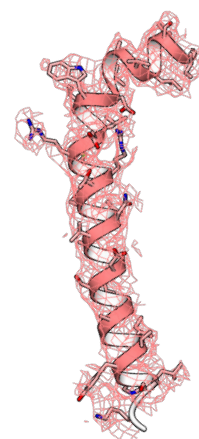
TM3



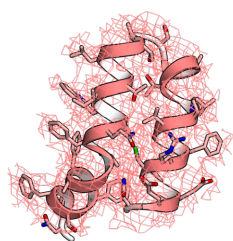
TM6



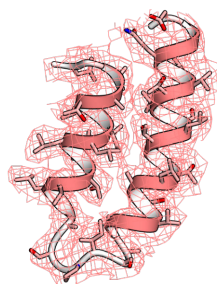
TM7



TM8



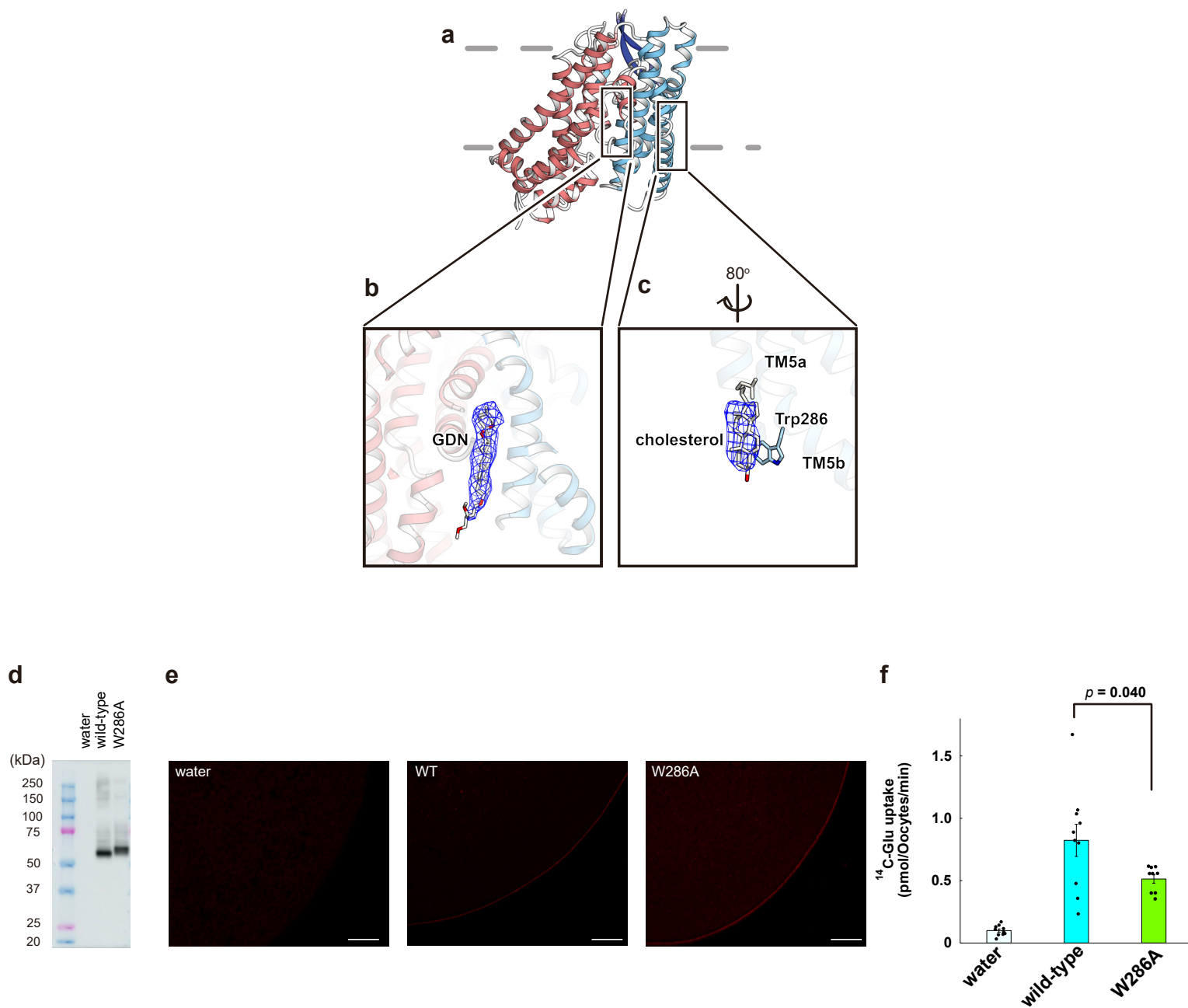
HP1



HP2

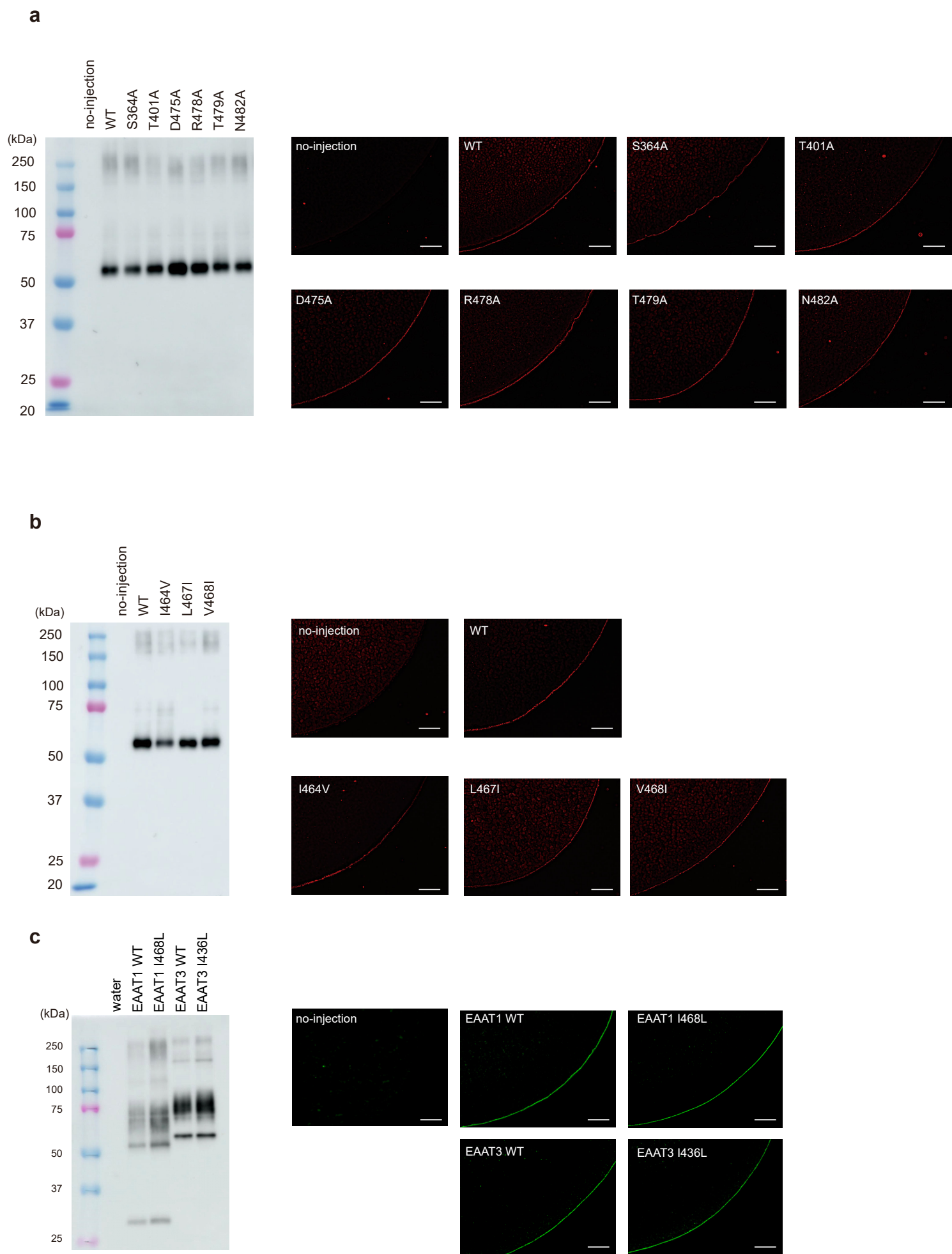
Supplementary Figure 4 | Atomic model of HsEAAT2 in the cryo-EM map

(a, b) Densities of all transmembrane helices and helical hairpins (countoured at 1.0σ). **a**, The densities of the scaffold domain, and **b**, the densities of the transport domain and helical hairpins (HP1 and HP2). TM2, TM5, TM7 and TM8 are each divided into two segments.



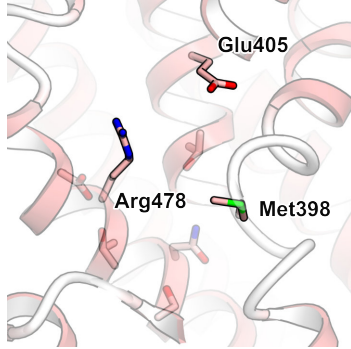
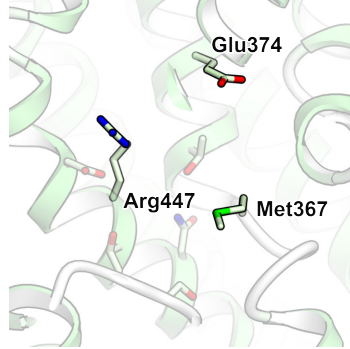
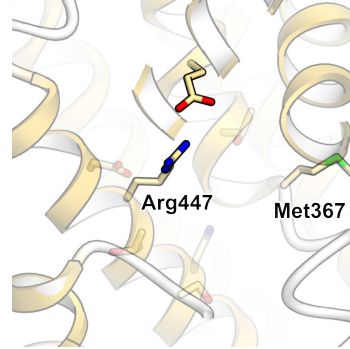
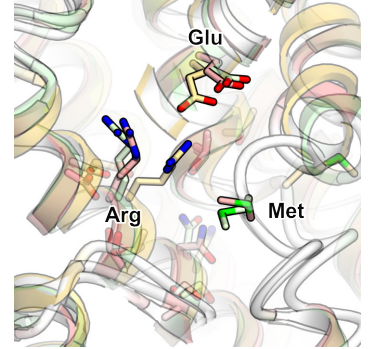
Supplementary Figure 5 | Lipid densities of the HsEAAT2 protomer

a, Overall structure of the HsEAAT2 protomer, viewed from the same orientation as in Fig. 2a. **b**, **c**, Close-up views of lipid densities. **b**, Density located between the transport domain and the scaffold domain. **c**, Density located at the scaffold domain. $F_o - F_c$ omit maps of both GDN and cholesterol are contoured at 2.5σ (normalized within mask), and shown as a blue mesh. **d**, **e** Western blotting and localization (scale bars = $50 \mu\text{m}$) of wild type and W286A. **f**, Glutamate uptake of control ($n = 10$), wild type ($n = 10$) and W286A ($n = 9$). Values are mean $\pm$ s.e.m. (P value is from two-side t test. $*p = 0.040$. 95% confidence interval).

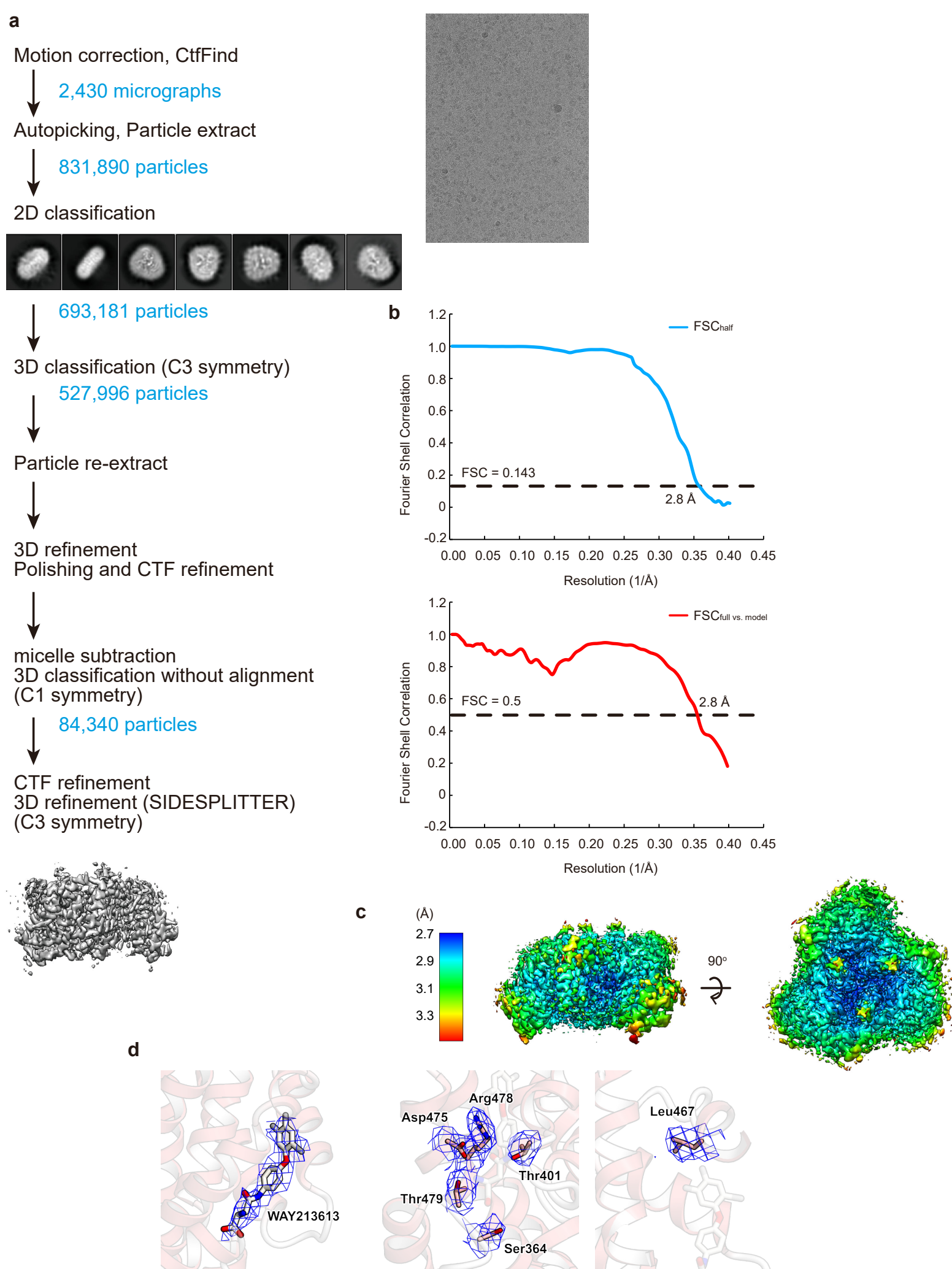


Supplementary Figure 6 | Expression level and localization of each mutant

Expression level and localization for **a)** each mutant of the substrate-binding site, **b)** each mutant of the cavity and **c)** mutants of EAAT1 and EAAT3. The western blotting analysis and the fluorescence detection (scale bars = 50 μ m) at the plasma membrane are shown on the left and right sides, respectively. All measurements were repeated at least three times, and one representative result for each mutant is shown.

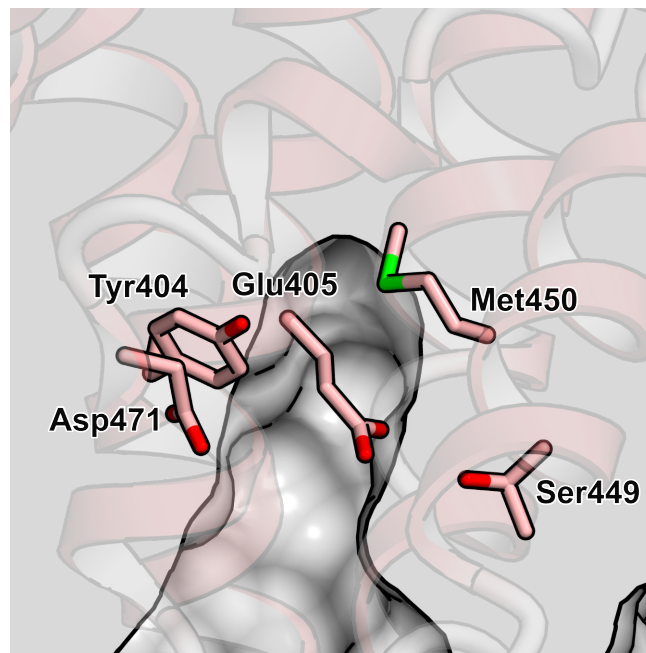
EAAT2**EAAT3 IFS- Na^+** **EAAT3 apo-state****molecular superposition****Supplementary Figure 7 | Comparison between EAAT2 and EAAT3**

Close-up views of the pockets of EAAT2 substrate-free state, EAAT3 apo-state (PDB ID 6X3F) and EAAT3 IFS- Na^+ (6X2Z), and their molecular superposition. Opaque sidechains indicate residues recognizing aspartate and glutamate at the binding site.



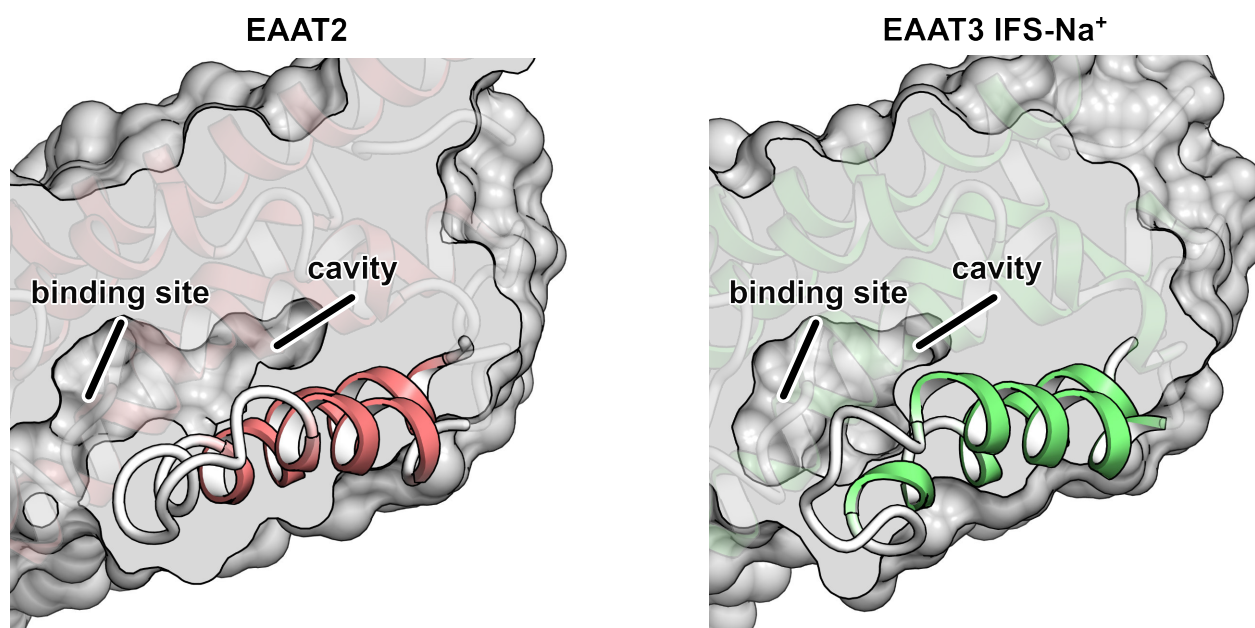
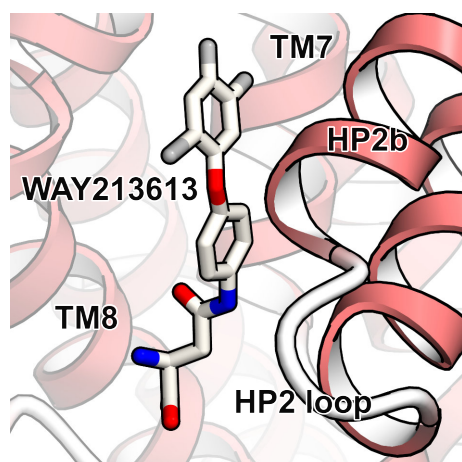
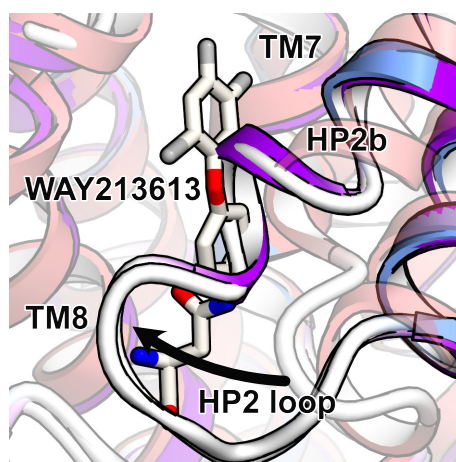
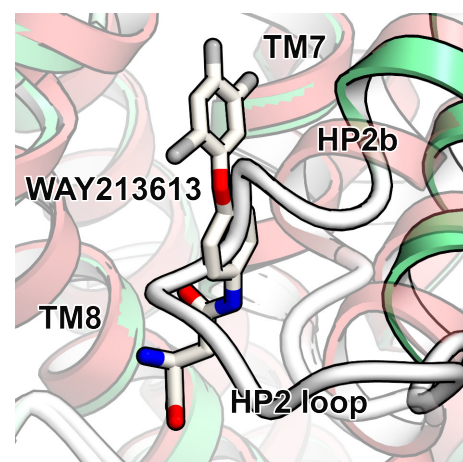
Supplementary Figure 8 | cryo-EM analysis of the HsEAAT2 IFS-WAY213613 state

a, Flow chart of cryo-EM data processing of the HsEAAT2 IFS-WAY213613 state. **b**, Fourier Shell Correlation (FSC) curve of the final 3D reconstruction model calculated using "relion_postprocess" with masked marked 2.8 Å resolution, corresponding to the FSC = 0.143 gold standard cut-off criterion (blue). FSC curve of map vs. model (red). **c**, Local resolution of the HsEAAT2 IFS-WAY213613 state. **d**, Densities of WAY213613 (left panel), residues recognizing the LA moiety (centre panel) and Leu467 (right panel).



Supplementary Figure 9 | Conserved residues at the cavity

Close-up view of the cavity formed near the substrate-binding site. Five residues (Tyr404, Glu405, Ser449, Met450 and Asp471) are completely conserved among EAATs.

a**b** **EAAT2 IFS-WAY213613****c****d**

Supplementary Figure 10 | Structural comparison of transport domains between HsEAAT2 and other SLC1A transporters

a, Cut-away representations of transport domains. The slab thicknesses are the same. **b**, Close-up view of the WAY-213613 binding site. **c**, Molecular superpositions among the IFS-WAY213613 state and Asp-bound states of EAAT1 (purple and PDB ID 5LLU) and EAAT3 (light blue and 6X2Z). The arrow indicates the movement of the HP2 loop. **d**, Molecular superpositions between IFS-WAY213613 and TFB-TBOA bound state of EAAT1 (green and PDB ID 5MJU).

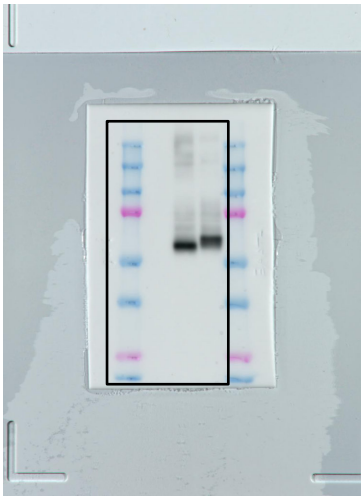
Cryo-EM data collection, refinement and validation statistics

	HsEAAT2 substrate-free state (EMDB-32098) (PDB 7VR8)	HsEAAT2 IFS-WAY213613 (EMDB-32097) (PDB 7VR7)
Data collection and processing		
Magnification	× 105,000	× 105,000
Voltage (kV)	300	300
Electron exposure (e-/Å ²)	50	50
Defocus range (μm)	-0.8 to -1.6	-0.8 to -1.6
Pixel size (Å)	0.83	0.83
Symmetry imposed	C3	C3
Initial particle images (no.)	1,090,865	831,890
Final particle images (no.)	91,390	84,340
Map resolution (Å)	3.2	2.8
FSC threshold	0.143	0.143
Refinement		
Initial model used (PDB code)	6GCT	
Model composition in the asymmetric unit		
Non-hydrogen atoms	3,012	3,012
Protein residues	402	402
Ligands ^a	4	6
Average B factors (Å ²)		
Protein	173.8	88.9
Ligand	221.4	143.0
R.m.s. deviations		
Bond lengths (Å)	0.017	0.015
Bond angles (°)	2.05	2.02
Validation		
MolProbity score	2.45	2.52
Clashscore	9.95	9.62
Poor rotamers (%)	5.79	7.01
Ramachandran plot		
Favored (%)	94.92	94.67
Allowed (%)	5.08	7.5.33
Outlier (%)	0	0

^a Ligands include cholesterol, head moiety of GDN, phospholipid and WAY213613

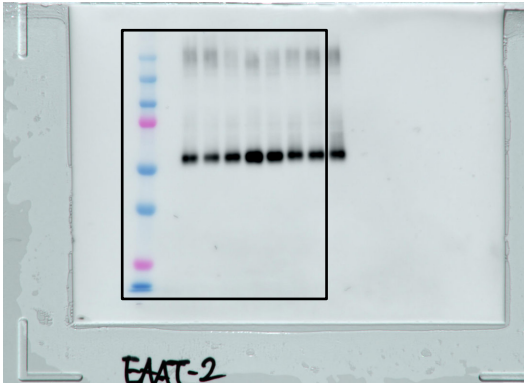
Uncropped scans of blots in Supplementary Figures

Supplementary Figures 5d

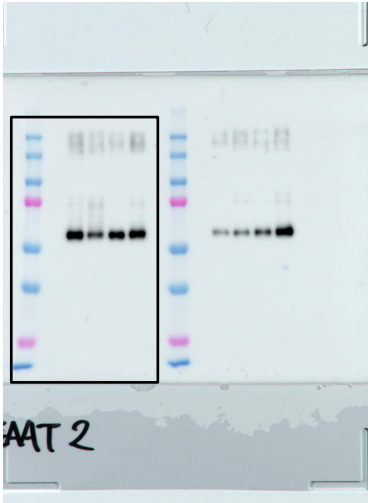


Supplementary Figures 6

a



b



c

