Supplementary information

Substrate binds in the S1 site of the F253A mutant of LeuT, a neurotransmitter sodium symporter homolog

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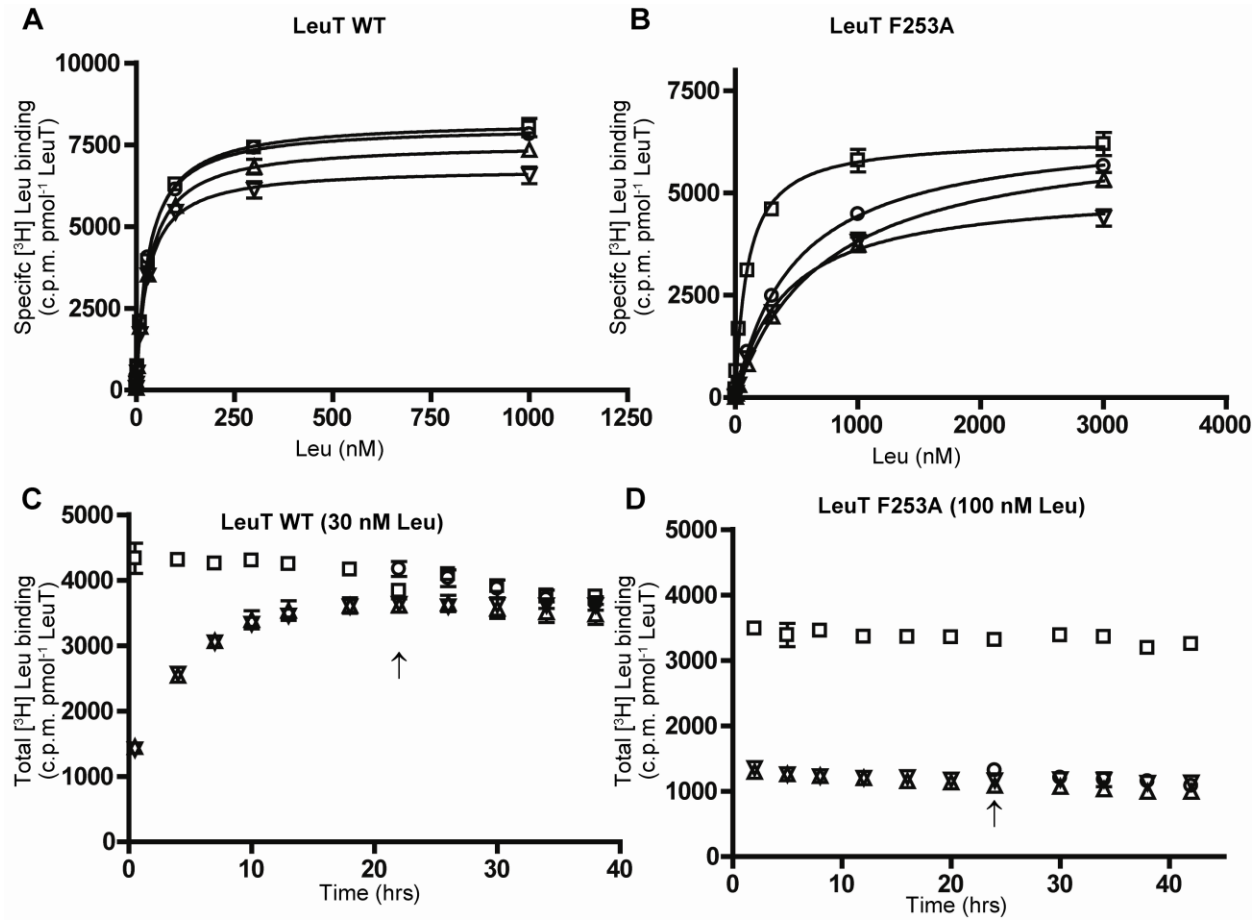
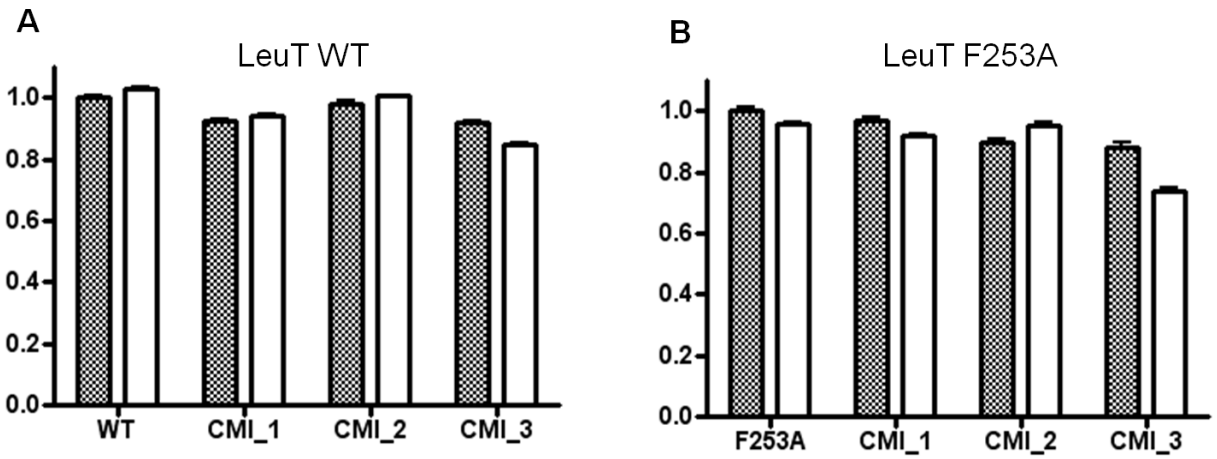
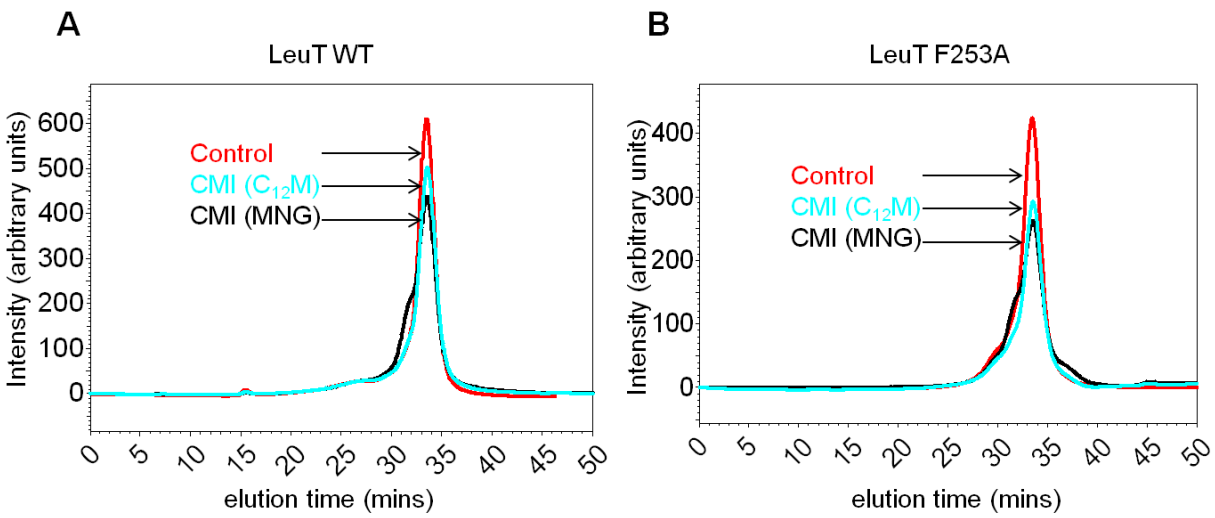
Table of contents



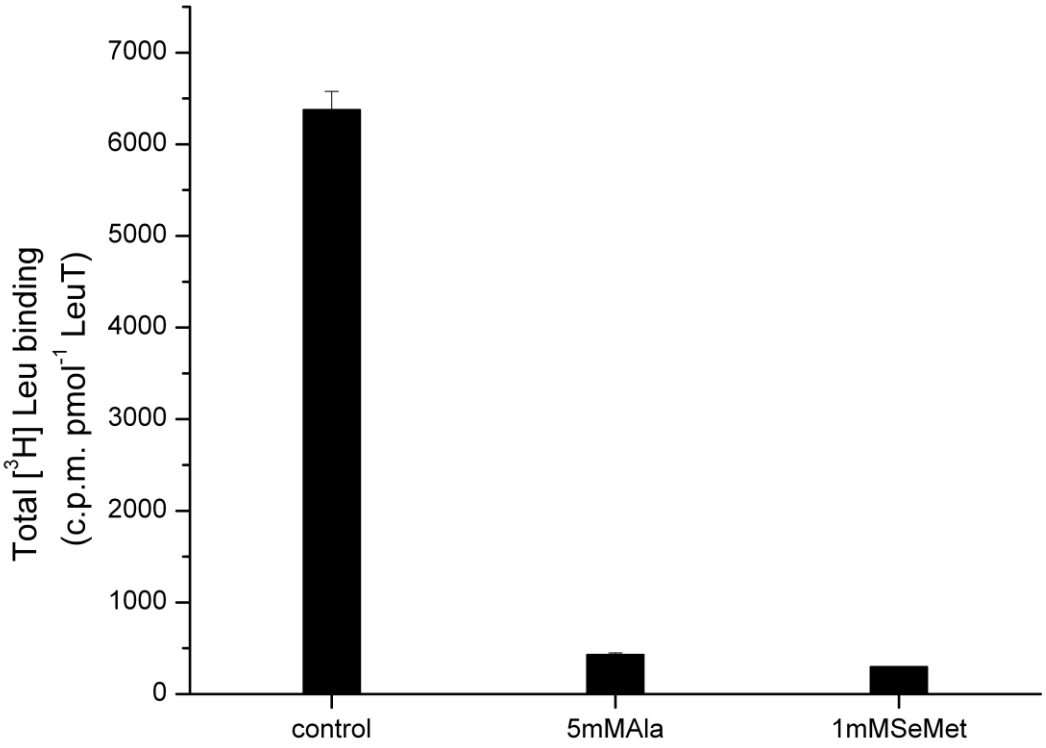
Supplementary Fig S1, Measurement of ^{3}H-leucine binding to wild type LeuT (LeuT WT) and to the F253A mutant by scintillation proximity assays (SPA) in the detergent $C_{12}M$. Presented are saturation binding isotherms and nonlinear regression analysis for LeuT WT (**A**) and for the LeuT F253A mutant (**B**), without CMI (open squares) and with 1 mM CMI. CMI was added either before leucine (inverted triangle), simultaneously with leucine (triangle) or after equilibrium is achieved (circle). Nonspecific binding was measured by binding in the presence of 5 mM Ala. Data are fit to a single exponential. Plot for total binding of 30 nM Leu to LeuT WT (**C**) and 100 nM Leu to LeuT F253A (**D**) without CMI and with 1 mM CMI as a function of time. The time points at which CMI was added are labeled by arrows. Symbols are as in (A) (B). Error bars, s.e.m.; n = 6-9.



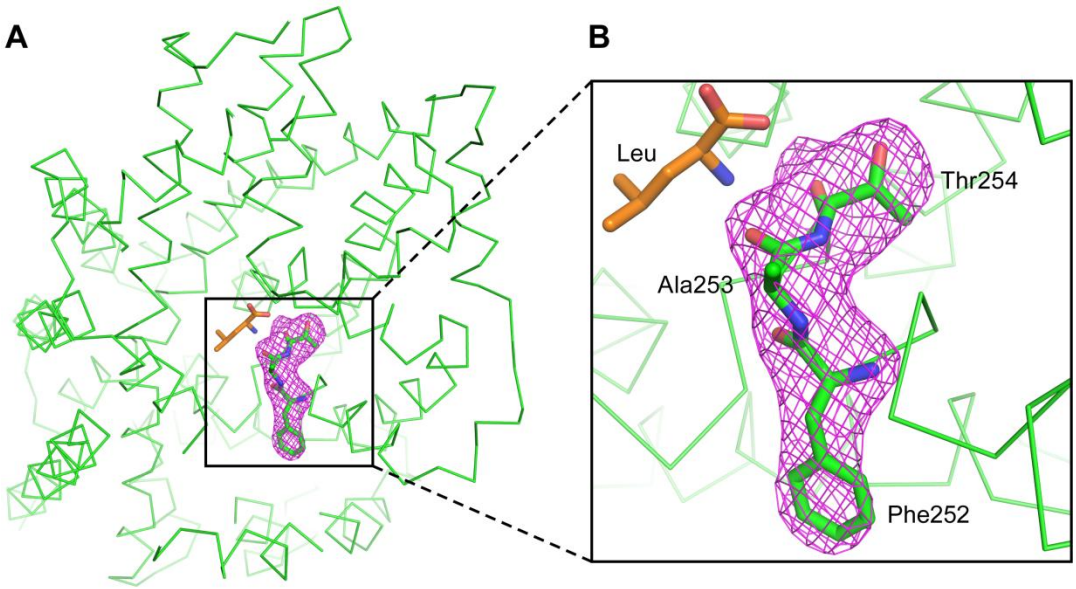
Supplementary Fig S2, The inclusion of CMI in ^{3}H-leucine saturation binding experiments does not dramatically decrease the total binding of leucine to LeuT WT and to the F253A mutant. Bar graphs of the B_{max} data derived from Supplementary Figure S1. Relative B_{max} values for leucine binding to LeuT WT (**A**) and to the F253A mutant (**B**). The columns labeled 'WT' (A) and 'F253A' (B) are for binding experiments in carried out in the absence of CMI. Additional experiments are where CMI was added at the same time as leucine (CMI_1), where CMI was added after 23hrs of incubation with ^{3}H leucine (CMI_2) and where CMI was added 1 hr before the addition of ^{3}H leucine (CMI_3). The shaded bars are for experiments carried out in MNG and the open bars are for experiments performed in $C_{12}M$. Error bars, s.e.m.; n = 3-6.



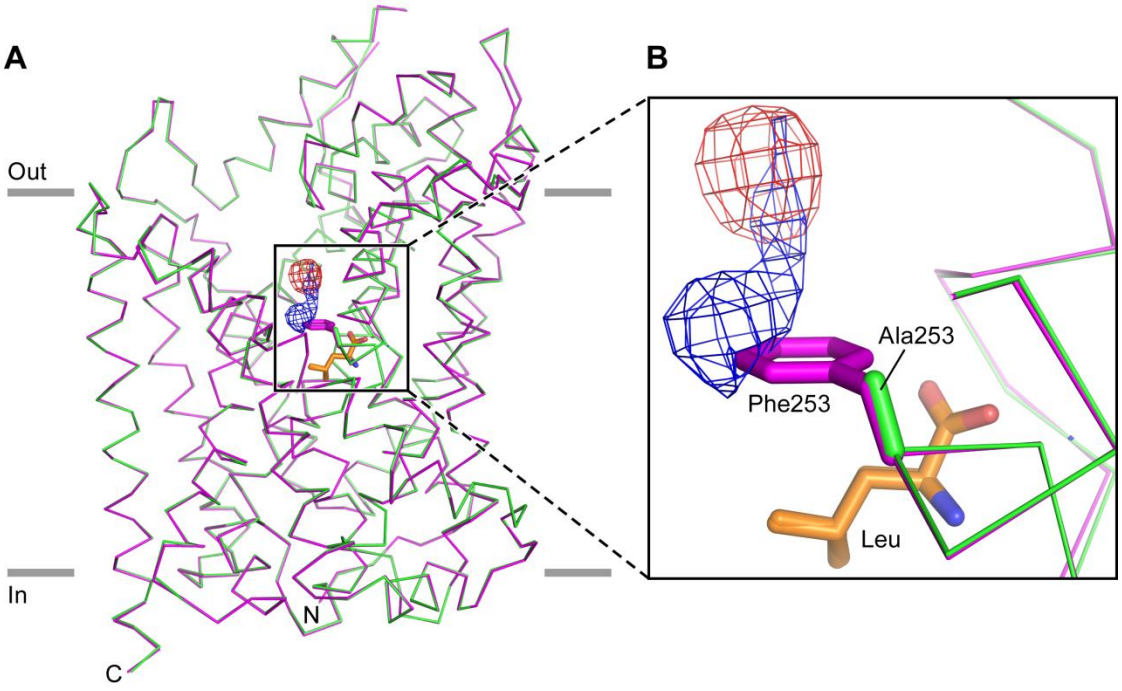
Supplementary Fig S3, Incubation of LeuT with CMI diminishes protein concentration as measured by fluorescence-detection size-exclusion chromatography (FSEC). Shown are FSEC traces for LeuT WT (**A**) and for the LeuT F253A mutant (**B**) in the absence (color red) and presence of CMI and in buffers containing either $C_{12}M$ (dodecyl maltoside) detergent (color cyan) or MNG-3 (lauryl maltose neopentyl glycol) detergent (color black). Fluorescence was measured by excitation at 295 nm and detection at 335 nm.



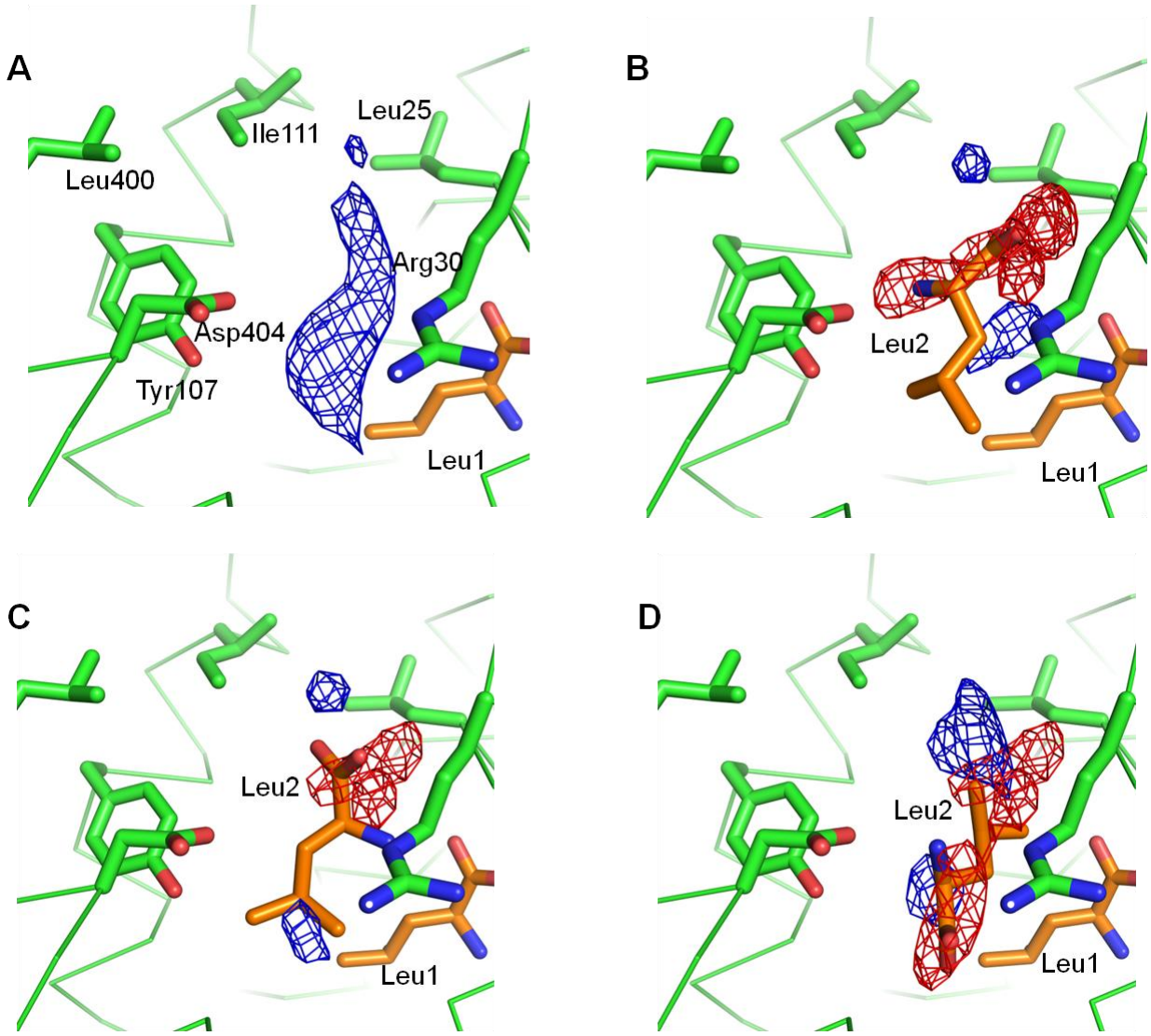
Supplementary Fig S4, Alanine (Ala) and selenomethionine (SeMet) compete for ^{3}H leucine binding to the LeuT F253A mutant. Here we measured the binding of 100 nM ^{3}H leucine to the LeuT F253A mutant in the absence (control) or in the presence of 5 mM cold Ala or 1 mM cold SeMet. Error bars, s.e.m.; n = 3.



Supplementary Fig S5, 'Omit' electron density difference map for residues Phe252, Ala253 and Thr254 in leucine complex of the LeuT F253A mutant. The view is from extracellular vestibule. (**A**) The Fo-Fc difference electron density map is displayed at 3σ, depicted in purple mesh and calculated with Phe252, Ala253 and Thr254 omitted from the structure factor calculation. Leu (orange) is in the primary site and shown in stick representation. (**B**) Close-up of the omit map.



Supplementary Fig S6, Superposition of the WT LeuT-Leu structure (PDB code 3USG; crystallized in bicelles; color magenta) on the structure of the leucine complex of the LeuT F253A mutant structure shows that the structures are similar within experimental error. (**A**) Residual Fo-Fc electron density features at the base of the extracellular vestibule for the previously reported WT LeuT-Leu structure (red mesh) and for the leucine complex of the LeuT F253A mutant (blue mesh). Phe253 in the WT and Ala253 in the mutant are displayed in stick representation. The bound substrate, Leu (orange), occupies the primary or S1 site is shown in stick representation. (**B**) Close up of the residual density in the vestibule.



Supplementary Fig S7, A second leucine molecule does not fit the residual density at the base of the extracellular vestibule of the LeuT F253A mutant. (**A**) Residual positive electron density from an 'omit' style Fo-Fc difference map. (**B-D**) Residual electron density from Fo-Fc difference maps after leucine was modeled into the residual density in (**A**) in three different orientations and subject to crystallographic refinement. Red and blue meshes illustrate negative and positive peaks, respectively. The maps are contoured at 3σ.

Supplementary Table I. Saturation binding experiments for WT and F253A mutant of LeuT*

	WT	WT+ CMI[1]	WT+CMI[2]	WT+ CMI[3]
K_d (MNG)	22.8 ±1.0	17.7 ±0.9	19.0 ±1.1	23.7 ±1.2
B_{max}(MNG)	8044 ±82	7440 ±81	7873 ±98	7387 ±85
K_d (C_{12}M)	31.7 ±0.9	32.3 ±1.1	30.0 ±0.6	28.4 ±1.0
B_{max}(C_{12}M)	8241 ±74	7543 ±85	8065 ±49	6786 ±82

	F253A	F253A + CMI[1]	F253A+CMI[2]	F253A + CMI[3]
K_d (MNG)	406.7 ±33.5	431.8 ±21.4	310.7 ±13.4	462.5 ±40.4
B_{max}(MNG)	6905 ±175	6709 ±104	6205 ±101	6088 ±169
K_d (C_{12}M)	99.4 ±5.1	716.9 ±56.1	488.6 ±15.1	408.7 ±38.7
B_{max}(C_{12}M)	6609 ±66	6332 ±76	6564 ±183	5102 ±149

* K_d (nM), B_{max} (c.p.m.pmol^{-1} LeuT) values shown are mean ± s.e.m (n = 3-6).

[1] CMI was added at the same time as ^{3}H leucine.

[2] CMI was added after 23 hrs equilibration with ^{3}H leucine.

[3] CMI was added 1 hr before the addition of ^{3}H leucine.