SUPPORTING INFORMATION

LOCATION OF THE ANTIDEPRESSANT BINDING SITE IN THE SEROTONIN TRANSPORTER: IMPORTANCE OF SER438 IN RECOGNITION OF CITALOPRAM AND TRICYCLIC ANTIDEPRESSANTS.

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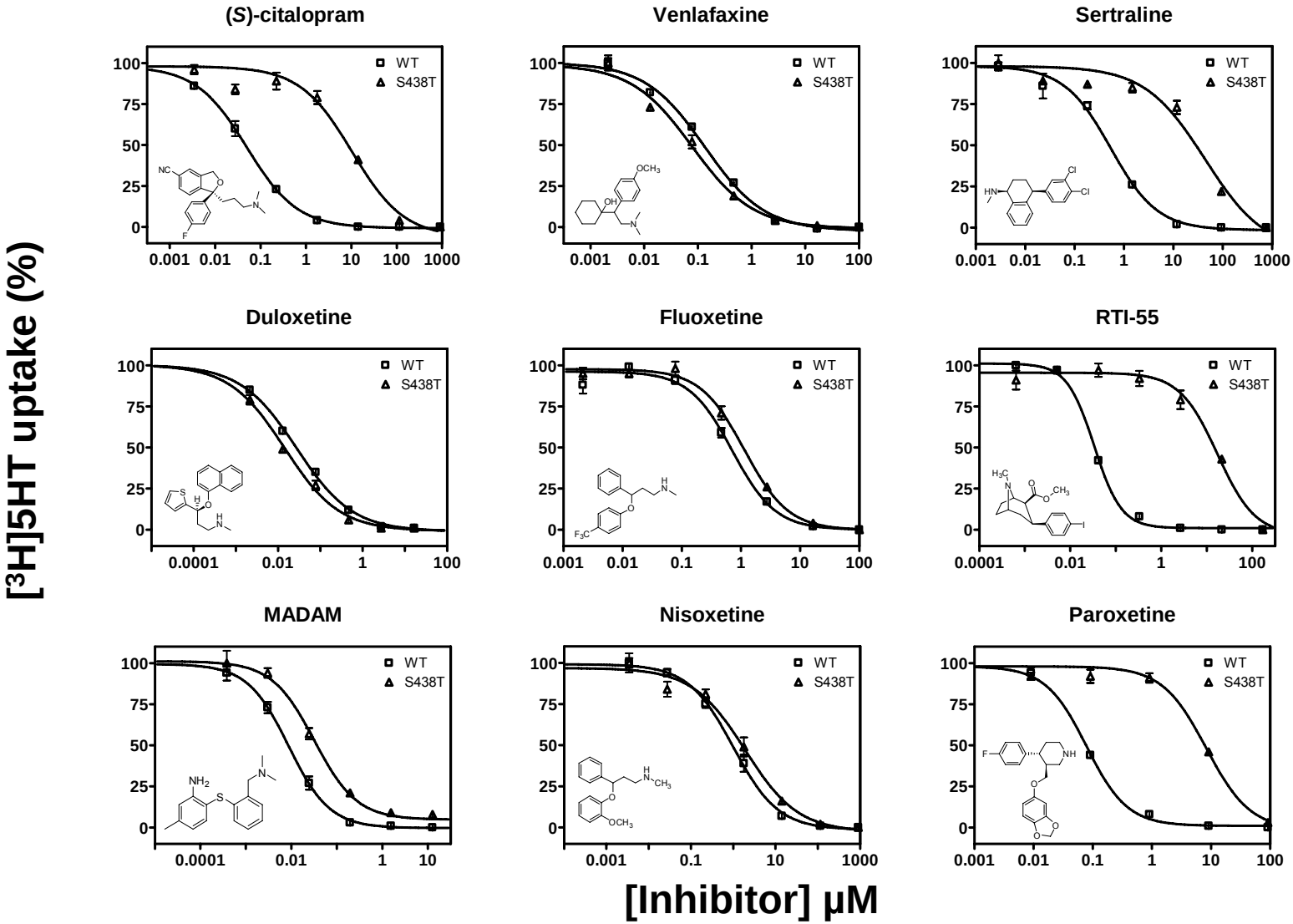
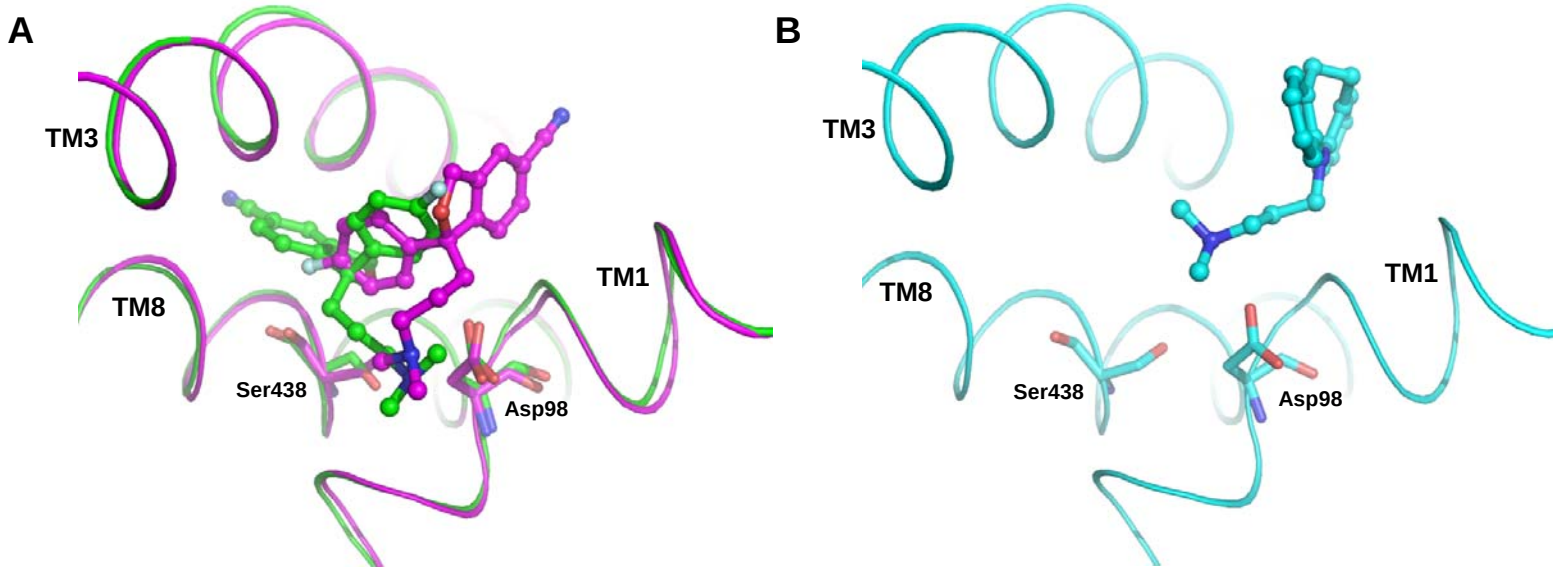
Supplementary Figure S1
Functional uptake inhibition analysis of SERT inhibitors at hSERT WT and hSERT S438T



COS-7 cells transiently expressing WT (□) or S438T (Δ) hSERT were incubated with 50 nM [^{3}H]-5HT and increasing concentration of inhibitor. After 30 min, nontransported radiolabelled 5HT was removed by rapid washing of wells with PBS. Accumulated [^{3}H]-5-HT was determined by scintillation counting. Resulting counts were normalized to percent uptake of control wells that lacked inhibitor.

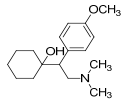
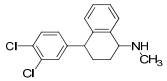
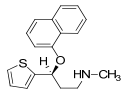
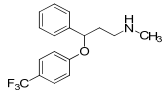
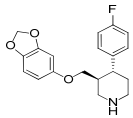
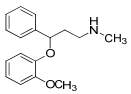
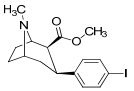
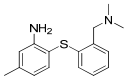
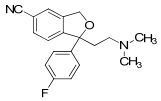
Supplementary Figure S2
Induced fit docking of (*S*)-citalopram and imipramine



A. Two different dockings of (*S*)-citalopram were carried out using the induced fit docking (IFD) in Maestro (Schrödinger, LLC, version 8.5). In the first docking, default settings in the IDF workflow were applied to dock (*S*)-citalopram into the binding cavity of the SERT WT protein (green model). Since the side chain of Phe335 closes the cavity, the option in the IFD workflow, in which this residue initially is mutated to an Ala residue and is added again later in the refinement, was used in a second IFD docking of (*S*)-citalopram (magenta model). The highest scoring binding modes of (*S*)-citalopram in complex with SERT from the two IFDs are shown. Without further experimental validation, it is impossible to distinguish between the two models (such studies are currently ongoing in our laboratories). The architecture of the binding sites is almost identical in the two models. The amino group of (*S*)-citalopram is deeply burried in the binding site in both models, but the two ring systems bind differently. Importantly, the location of the amino group is almost identical in the two models being in close proximity of Asp98 and Ser438. While we cannot distinguish between the two models, they agree that the amino group of (*S*)-citalopram is in close proximity (~4Å) of Ser438.

B. Two different IFDs of imipramine were carried out using the same approaches as described for (*S*)-citalopram. When docking imipramine into the cavity of SERT WT, no binding mode of imipramine, where the amino group had contact to Asp98, was found. In contrast, using the option of initially mutating Phe335 to an Ala residue, as described for (*S*)-citalopram, the highest scoring binding mode had a contact between the amino group of imipramine and the carboxylate group of Asp98 (model shown). The amino group of imipramine is not burried as deeply in the binding pocket as (*S*)-citalopram, most likely due to the more bulky ring system. This actually leaves space for a water molecule to bind favourably in the pocket (at -13 kcal/mol estimated with the Grid program). Ser438 is part of those residues defining the cavity, but is located somewhat further away from imipramine than (*S*)-citalopram (6Å vs. 4Å). Therefore, one might speculate that this is why imipramine is less influenced by the S438T mutation than S-citalopram is.

Supplementary Table S1. Chemical structure and inhibition constants for inhibitors at hSERT WT and hSERT S438T

Compound	Structure	hSERT WT K_i (nM)[a]	n	hSERT S438T K_i (nM)[a]	n	P (paired t test)[b]	Affinity change K_i(S438T)/K_i(WT)[c]
Venlafaxine		112.9 ± 15.5	6	53.0 ± 6.9	6	0.0029	0.5 ± 0.1
Sertraline		377.8 ± 69.3	6	30612.0 ± 4601.8	6	0.0012	90.8 ± 15.8
Duloxetine		55.8 ± 10.1	6	20.3 ± 4.3	6	0.0025	0.3 ± 0.0
Fluoxetine		512.0 ± 84.9	6	611.0 ± 75.5	6	0.049	1.2 ± 0.1
Paroxetine		38.3 ± 10.4	8	6339.2 ± 632.0	8	<0.0001	339.2 ± 113.0
Nisoxetine		795.0 ± 105.1	6	1442.2 ± 121.5	6	0.0005	1.9 ± 0.2
RTI-55		39.4 ± 6.3	8	16953.8 ± 2074.7	8	<0.0001	449.5 ± 33.7
MADAM		8.1 ± 0.9	6	17.9 ± 4.5	6	0.049	2.1 ± 0.4
Aminoethyl citalopram		260.1 ± 87.3	6	319.7 ± 58.6	6	0.11	1.6 ± 0.2

[a] Data represents mean ± S.E.M. [b] hSERT K_i compared with hSERT S438T K_i using paired t test. [c] K_i(S438T)/K_i(WT) determined from paired observations.
Data represents mean ± S.E.M.

Supplementary Table S2. Mutagenesis of vestibule residues in hSERT

	5-HT		(*S*)- Citalopram				Imipramine				Clomipramine					
Mutant	K_M (μM)[a]	n	K_i (nM)[a]	n	K_i(WT)/K_i(mutant)[b]	n	K_i (nM)[a]	n	K_i(WT)/K_i(mutant)[b]	n	K_i (nM)[a]	n	K_i(WT)/K_i(mutant)[b]	n	Transport activity[c]	n
I179D	N.F.		N.F.				N.F.				N.F.				N.F.	
I179F	0.60 ± 0.30	3	4.79 ± 0.89	9	0.77 ± 0.16	9	5.37 ± 1.3	9	0.22 ± 0.08 *	9	13.9 ± 2.12	9	0.60 ± 0.09	9	0.07 ± 0.01	29
D400F	0.18 ± 0.03	3	4.86 ± 0.68	8	0.59 ± 0.11	8	19.26 ± 3.5	8	0.43 ± 0.06 *	8	20.3 ± 1.83	8	0.58 ± 0.08 *	8	0.37 ± 0.04	23
D400K	0.12 ± 0.03	3	1.66 ± 0.23	8	0.27 ± 0.07 *	8	6.93 ± 2.4	8	0.18 ± 0.06 *	8	5.2 ± 0.47	9	0.26 ± 0.07 *	9	0.21 ± 0.03	27
D400L	0.60 ± 0.30	3	4.79 ± 0.89	9	0.40 ± 0.06 *	9	12.78 ± 1.7	9	0.36 ± 0.08 *	9	10.1 ± 1.22	10	0.49 ± 0.09 *	10	0.23 ± 0.03	30
L406D	N.F.		N.F.				N.F.				N.F.				N.F.	
L406F	3.01 ± 1.03	3	7.85 ± 0.51	6	0.85 ± 0.07	6	30.25 ± 4.1	6	0.71 ± 0.13	6	23.5 ± 2.67	6	1.06 ± 0.27	6	0.63 ± 0.04	18
L406K	N.F.		N.F.				N.F.				N.F.				N.F.	
V489D	N.F.		N.F.				N.F.				N.F.				N.F.	
V489F	0.63 ± 0.43	3	4.82 ± 0.85	6	0.59 ± 0.07 *	6	14.23 ± 2.2	7	0.48 ± 0.12	7	15.7 ± 1.81	7	0.84 ± 0.19	7	1.10 ± 0.09	20
V489K	N.F.		N.F.				N.F.				N.F.				N.F.	
K490D	0.16 ± 0.02	4	3.50 ± 0.60	8	0.38 ± 0.05 *	8	12.41 ± 2.5	7	0.36 ± 0.03 *	7	14.9 ± 3.27	8	0.42 ± 0.06 *	8	0.61 ± 0.04	25
K490F	0.17 ± 0.06	3	8.79 ± 0.79	6	0.99 ± 0.23	6	38.47 ± 5.8	6	0.80 ± 0.05	6	33.9 ± 4.34	6	0.85 ± 0.15	6	1.09 ± 0.04	18
K490T	0.27 ± 0.09	4	7.84 ± 0.94	6	1.06 ± 0.24	6	27.09 ± 3.1	6	0.75 ± 0.06	6	28.2 ± 3.81	7	0.71 ± 0.11	7	0.95 ± 0.05	22

[a] Data represents mean ± S.E.M. [b] K_i(mutant)/K_i(WT) determined from paired observations. Data represents mean ± S.E.M. [c] Activity of hSERT mutant compared to WT after incubation with 50 or 150 nM [^{3}H]-5HT for 30 min. Activity of WT is set to 1. * Significant change (P < 0.05) in K_i values. N.F.: Non-functional