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Supplementary Information for

Structural, Functional, and Behavioral Insights of Dopamine Dysfunction Revealed by a Deletion in SLC6A3

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This PDF file includes:

- Supplementary Information 1
- Supplementary Information 2
- Figs. S1 to S5
- Tables S1 to S2
- Captions for movies S1 to S2

Other supplementary materials for this manuscript include the following:

- Movies S1 to S2

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Supplementary Information I

Diagnosis: The autism-affected male in this family of European ancestry was ascertained as part of the Simons Simplex Collection, which recruits families comprising of a proband, unaffected parents and an unaffected sibling. The proband received a consensus diagnosis of "autism" on the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) classifications (Supplemental Figure S1). Cognitive testing indicated the proband had an IQ in the normal range (Full scale IQ: 94).

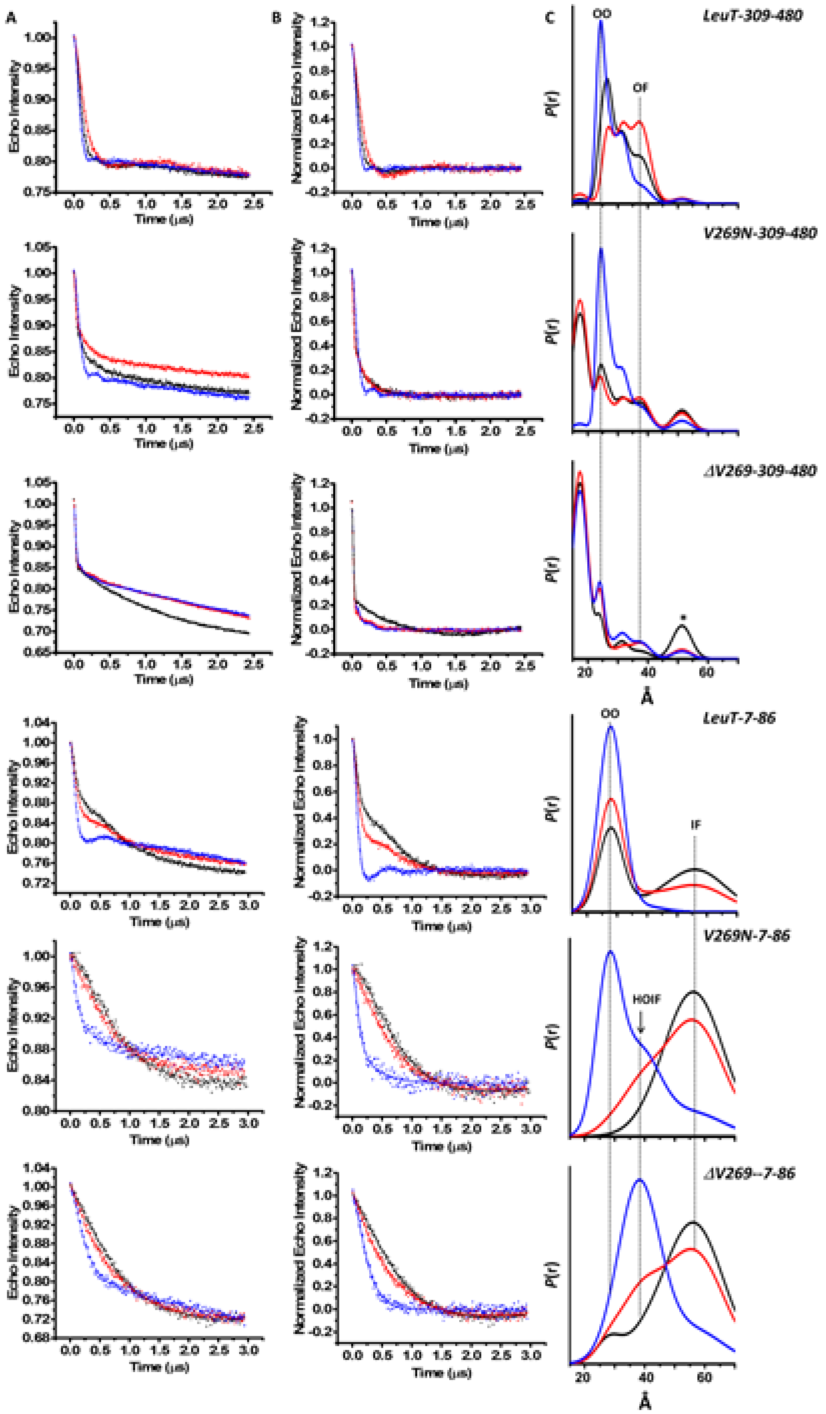
Scoring: Parsing ADI-R domains revealed deficits across all four categories: (1) Reciprocal Social Interaction (score=30; cutoff=10); (2) Abnormalities in Communication (score=22; cutoff=8); (3) Restricted, Repetitive and Stereotyped Patterns of Behavior (score=8; cutoff=3); (4) Development Evident at or Before 36 Months (score=1; cutoff=1).

ADOS testing was performed at 191 months using module 3, which is intended for verbally fluent children. The proband received a raw ADOS score of 27, and a calibrated severity score (CSS), a metric to approximate autism severity (Gotham et al., 2009), of 8. The average autism CSS score is 7.09 ± 2.45 (Gotham et al., 2009).

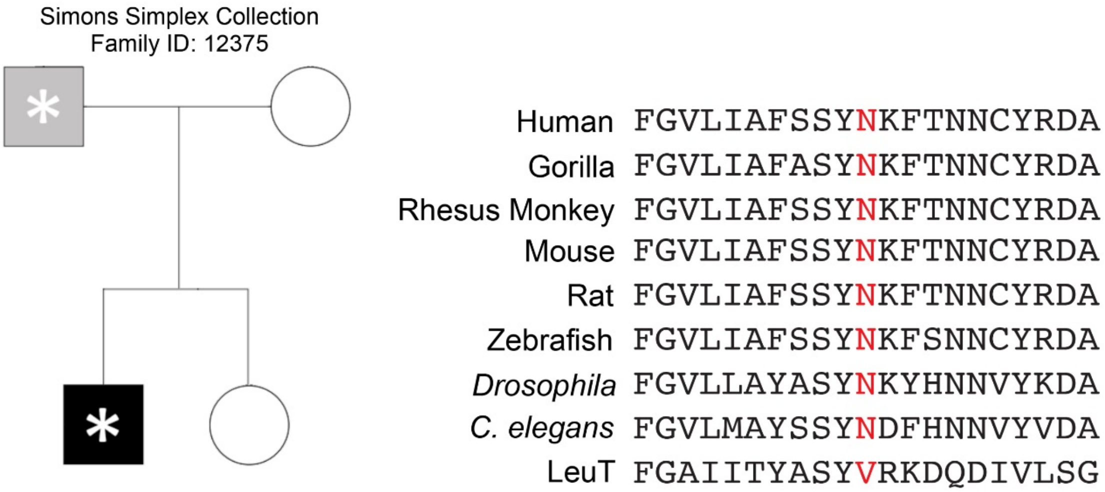
The Social Responsiveness Scale (SRS) is an instrument used to quantify social impairment across entire range of the autism spectrum and can be completed by parents and/or teachers. The proband received T-scores of 89 and 77 from the parents and teacher respectively, scores indicating "severe" deficits in everyday social interactions. Female sibling assessment using the SRS indicated normal development.

The broad autism phenotype (BAP) describes a set of behavioral and language features/deficits which occur in individuals resulting from a milder expression of the underlying genetic liability for autism. One instrument to measure BAP in adults is the Broad Autism Phenotype Questionnaire (BAPQ). The BAPQ was designed to measure aloof personality, rigid personality, and pragmatic language deficits. In this family, the father received an overall BAPQ score of 3.58 (cutoff=3.15) indicating a broad autism phenotype (Supplemental Figure S1), with BAPQ subscale scores of Aloof=3.33 (cutoff 3.25), Rigid=3.25 (cutoff 3.50), and Pragmatic Language=3.25 (cutoff 2.75). The mother of the proband received an overall score of 2.22 (cutoff=3.15) indicating no broad autism phenotype, with BAPQ subscale scores of Aloof=2.25 (cutoff 3.25), Rigid=2.25 (cutoff 3.50), and Pragmatic Language=2.17 (cutoff 2.75).

Supplementary Information 2

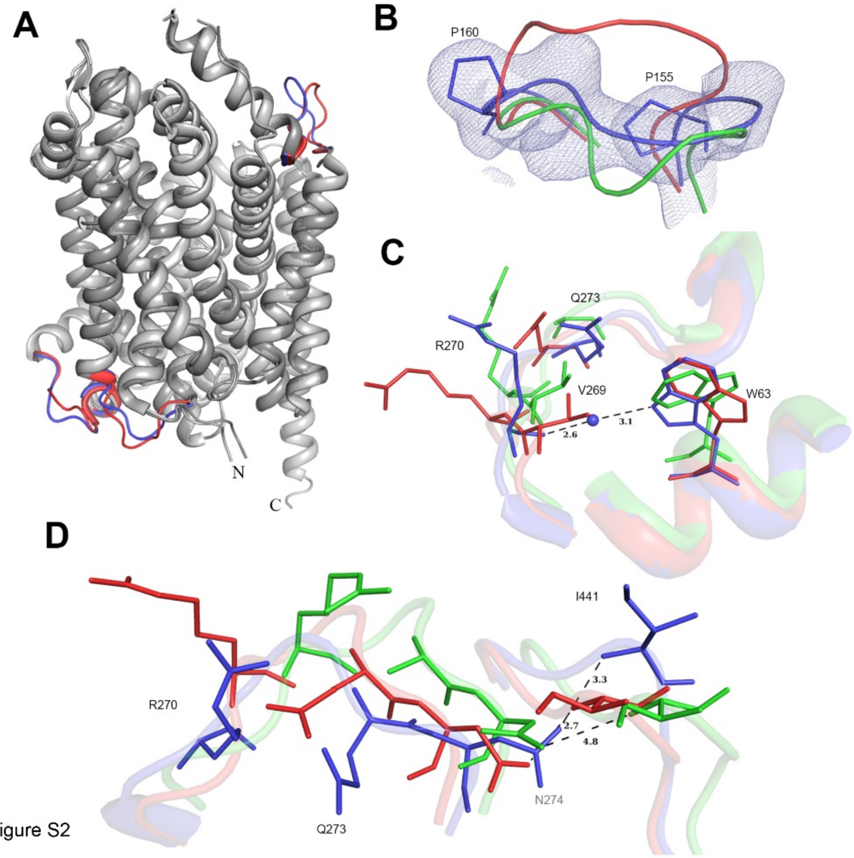


SI 2. DEER analysis of the mutants. (A) Primary DEER signal and associated fits, (B) normalized and background-corrected signals and (C) distance distribution P(r).



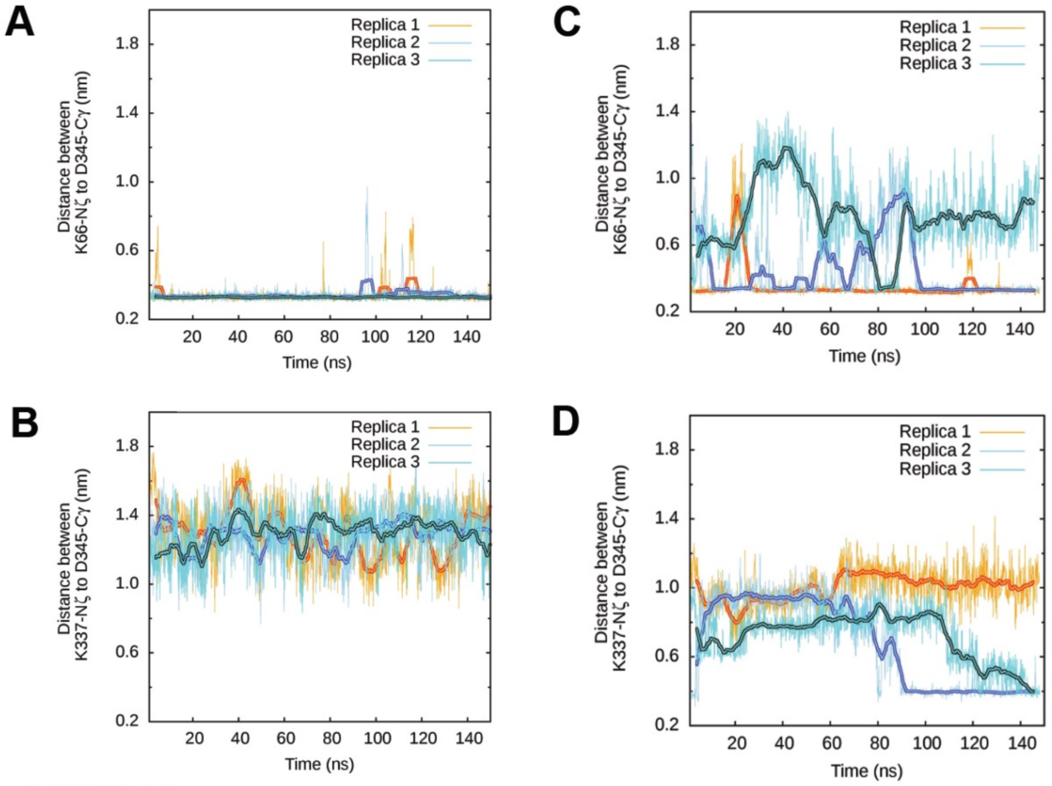
Supplemental Figure S1

Fig. S1. Identification of an in-frame deletion of N336 in hDAT in an ASD family. Left: An inherited in-frame deletion was identified in an ASD family within the Simons Simplex Collection. Black filled boxes indicate affected individuals (diagnosed with ASD), and grey filled boxes indicate individuals testing broad autism phenotype on the BAPQ. Asterisks indicate individuals heterozygous for the variant. Right: High residue conservation across species at and surrounding the residue N336 (in red), predicts a functional effect of the variant.



Supplemental Figure S2

Fig. S2. Related to Figure 2. Structure of LeuT ΔV269. A: Differences in the regions other than the N-termini are highlighted in red (2A65) and blue (ΔV269). B: Movement of loop 153-162. The residues in ΔV269 are highlighted by a 2Fo-Fc density map. The same regions in 2A65 and 3TT3 (green) are displayed in cartoon representation in the background. C: Side chain flip of W63. The water molecule coordinating the indole ring is displayed as a blue sphere. D: Movement of loop 438-444. Residue I441 and N274 are highlighted.



Supplemental Figure S3

Fig. S3. Related to Figure 3. In hDAT, the ΔN336 promotes repositioning of K337 that weakens the K66-D345 interaction by competing for D345. A: Time evolution of distances in hDAT. Distances are measured between K66 atom Nζ, and D345 atom Cɣ. B: Time evolution of distances in hDAT. Distances are measured between K337 atom Nζ, and D345, atom Cɣ. C: Time evolution of distances in hDAT ΔN336. Distances are measured between K66 atom Nζ, and D345 atom Cɣ. D: Time evolution of distances in hDAT ΔN336. Distances are measured between K337 atom Nζ, and D345, atom Cɣ. All simulations were conducted in triplicate from independent starting structures.

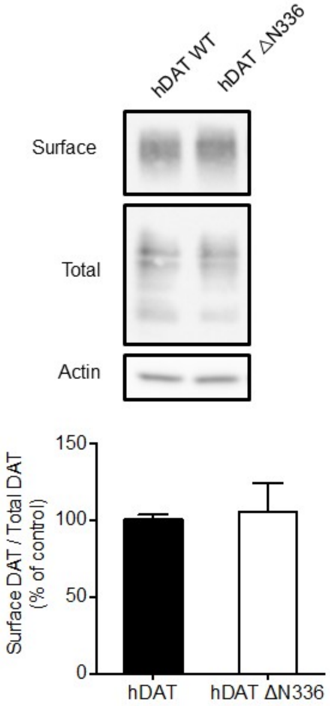
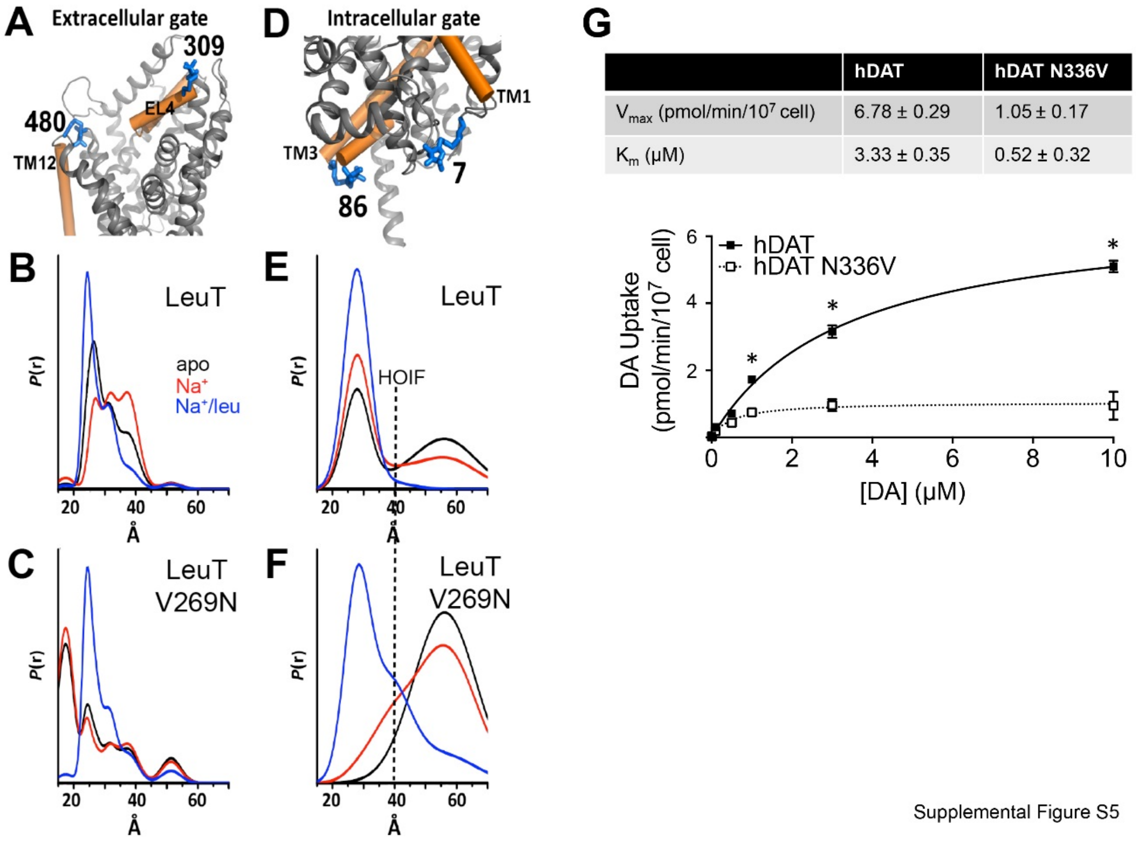


Figure S4

Fig. S4. Related to Figure 4. hDAT ΔN336 displays equivalent expression of total and surface protein. Representative immunoblots for biotinylated (surface) and total protein fractions from hDAT and hDAT ΔN336 cells. Surface fractions were quantitated, normalized to total DAT, and expressed a percent of control ($p \geq 0.05$ by Students t-test; $n = 4$). Actin serves as a loading control.



	hDAT	hDAT N336V
V_{max} (pmol/min/10^7 cell)	6.78 ± 0.29	1.05 ± 0.17
K_m (µM)	3.33 ± 0.35	0.52 ± 0.32

Supplemental Figure S5

Fig. S5. V269N increases the intracellular gate dynamics in LeuT. **A**: Extracellular reporter pairs (309-480) tagged on a three-dimensional structure of LeuT. **D**: Intracellular reporter pairs (7-86) tagged on a three-dimensional structure of LeuT. **B,E**: Distance of the extracellular and intracellular reporter pair respectively for LeuT, in the apo conformation (black), in the presence of Na+ (red), or in the presence of Na+ plus Leu (blue). **C,F**: Distance of the extracellular and intracellular reporter pair for LeuT V269N, in the apo conformation (black), in the presence of Na+ (red), or in the presence of Na+ and Leu (blue). **G**: Top: Kinetic parameters (Vmax and Km) for hDAT and hDAT N336V (n = 3, in triplicate). Bottom: Representative plot of [3H]DA uptake kinetics in hDAT (filled squares) or hDAT V336N (empty squares) cells (* = p<0.05 by two-way ANOVA followed by Bonferroni post test; n = 3, in triplicate).

Table S1. Related to Figure 2. Table representing the log likelihood gain score observed in phaser using various search models, which served as an indication of conformation of LeuT ΔV269 structure.

PDB ID	Conformation	LLG score from MR
3TT3	Inward open	199
3TT1	Outward open	2000
5JAE	Outward-oriented, Na+ free return state	2500
3USG	Outward-occluded, bound to L-leucine	6110
2Q72	Outward-occluded, L-leucine and Na+ bound	5700

Table S2. Related to Figure 2. Data collection, model building, and refinement statistics.

LeuT ΔV269	
Wavelength (Å)	1.000
Resolution range (Å)	64.05 - 2.62 (2.68 - 2.62)
Space group	C 2
Unit cell	149.54 86.14 81.53 90 110.721 90
Total reflections	85948 (5195)
Unique reflections	28540 (2042)
Multiplicity	3.5 (3.6)
Completeness (%)	96.9 (95.1)
Mean I/sigma(I)	8.8 (1.9)
Wilson B-factor (Å^2)	48.6
R-merge (%)	10.5 (41.3)
R-meas (%)	12.6 (51.1)
CC1/2 (%)	99.5 (60.1)
CC* (%)	99.8 (88.4)
Reflections used in refinement	28521 (2766)
Reflections used for R-free	1426 (138)
R-work (%)	21.56 (29.60)
R-free (%)	25.02 (34.61)
CC (work)	91.7 (74.5)
CC (free)	81.0 (76.6)
Number of non-hydrogen atoms	4000
macromolecules	3838
ligands	85
solvent	77
Protein residues	492
RMS (bonds) (Å)	0.003
RMS (angles) (°)	0.61
Ramachandran favored (%)	97.73
Ramachandran allowed (%)	2.27
Ramachandran outliers (%)	0
Rotamer outliers (%)	0.53
Clashscore	8.22
Average B-factor (Å^2)	57.29
macromolecules	56.78
ligands	80.59
solvent	56.86

Movie S1. Movie of a hDAT WT fly showing the short freezing period, followed by a distinctive and rapid increase in average velocity (fleeing) upon a predatory sound (red highlighting shade). The video frame/rate was reduced 4X.

Movie S2. Movie of a hDAT ΔN336 fly showing a prolonged freezing period, followed by a reduced increase in average velocity (fleeing) upon a predatory sound (red highlighting shade). The video frame/rate was reduced 4X.