

## Supplementary material

### **The effect of epileptogenic mutations on the function and trafficking of the GABA transporter GAT-1**

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**Figure S1.** Subcellular distribution of GAT-1 variants in transfected cells. COS7 cells were co-transfected with expression vectors for HA-GAT-1 WT or the indicated HA-tagged mutants- plus mCherry-DAT. 48 h later, cells were fixed and incubated with anti-HA and anti-mCherry primary antibodies followed by Alexa Fluor-labeled secondary antibodies. Images collected by confocal microscopy correspond to distribution of HA-GAT-1 variants (green channel), mCherry-DAT (red channel), or the merge. Scale bar: 10  $\mu$ m.

## **Figure S2**

Screenshot of the protein region around G307. **(A)**. In G307WT, a glycine located at the cytoplasmic end of TM6 makes polar and carbonyl and hydrogen bonds with adjacent residues (from L303 to Y309), as well as with G105 and Q106 in the cytoplasmic end of TM2. **(B)** In the mutant G307R, the lateral group of arginine has many novel interactions with TM2 (from E101 to I304) as well as with M487 located in the intracellular loop 5, IC5. Color code: clash, pink lines; hydrophobic, green lines; hydrogen bonds, red lines; carbonyl, dark blue; van der Waals, light blue. A detailed spin animation of this region is presented in supplementary video 1 (G307WT) and supplementary video 2 (G307R).

It can be appreciated that in G307WT, the peptide carboxyl and amino groups of glycine establish hydrogen bonds and polar interactions with the nearby residues L303, I304 and Y309, all in TM6b, and with G105 and Q106 in the cytoplasmic end of TM2. However, arginine, apart from these interactions, establishes a multitude of clash interactions through its lateral chain, especially with TM2. Moreover, charged amino groups from the R group are inserted into TM2 where they establish numerous additional polar interactions. Also, additional interactions are observed with N310 and M457 (IC5).

Figure S1

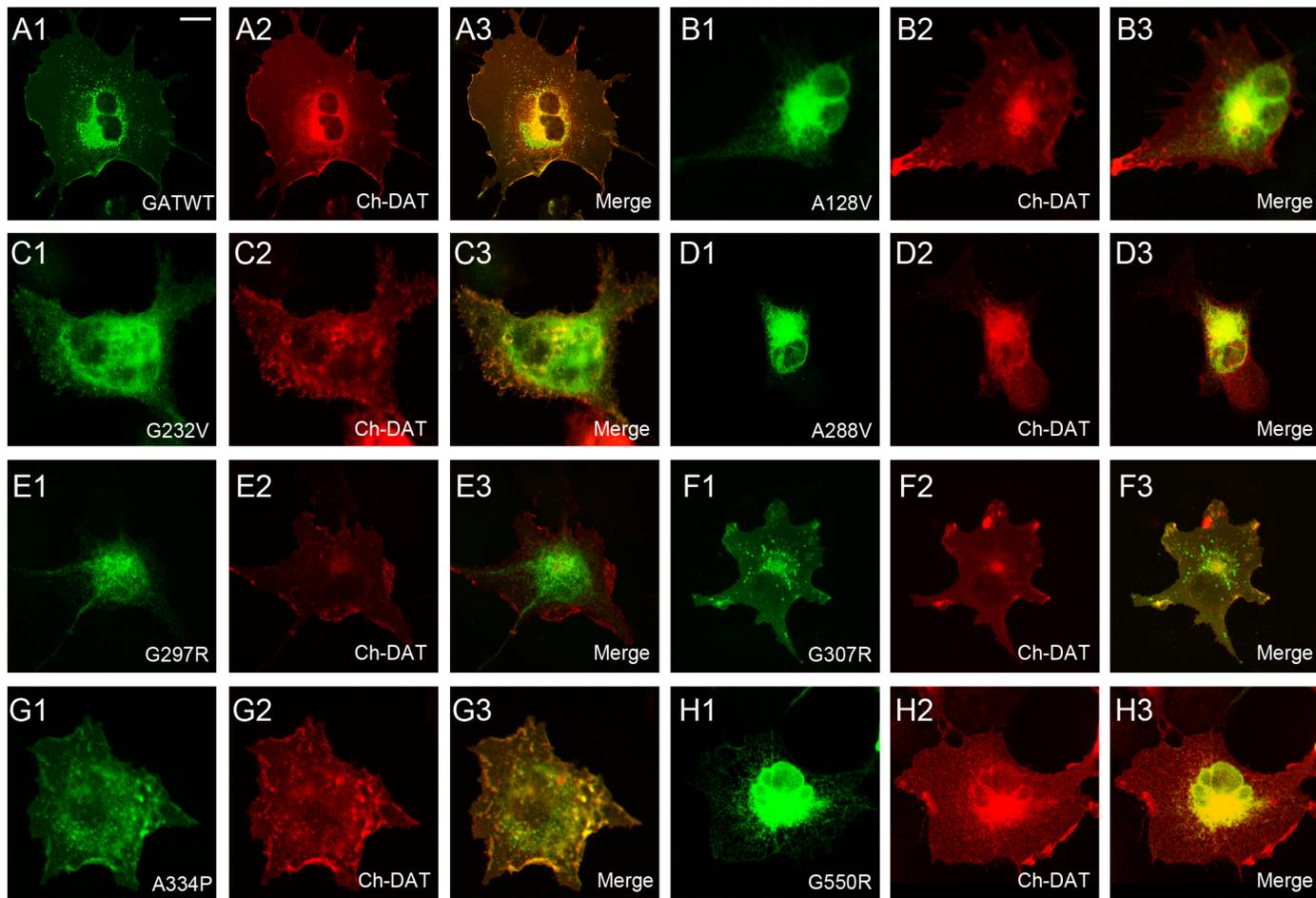


Figure S2

