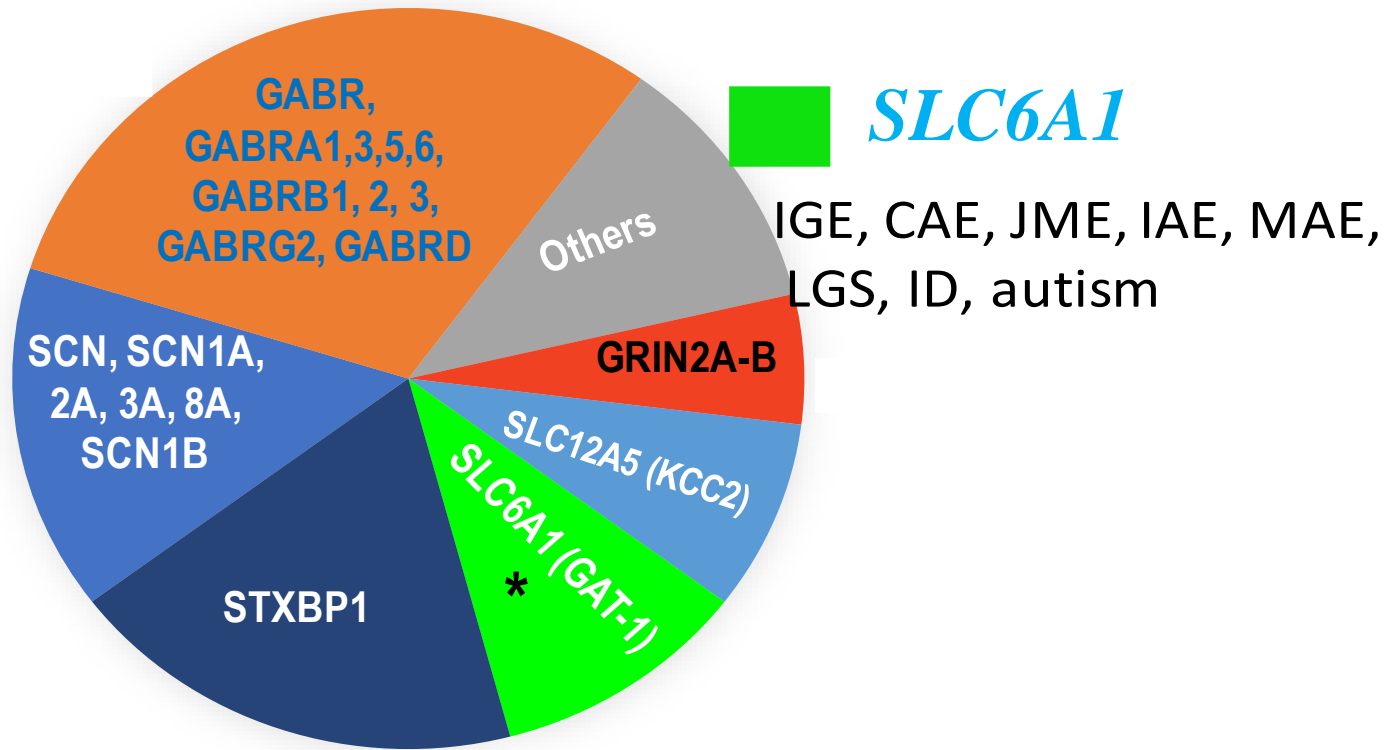


A photograph of the Vanderbilt University campus in autumn. The scene is dominated by rows of trees with vibrant yellow and orange leaves. In the background, a large, classical-style building with a prominent dome and a portico of columns is visible. The sky is a clear, pale blue. The overall atmosphere is bright and scenic.

Modeling SLC6A1 mutations from stem cell to mouse models

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Department of Neurology & Pharmacology
Vanderbilt Brain Institute
Vanderbilt University Kennedy Center of Human
Development
Vanderbilt University Medical Center
Nashville, TN, 37232

Mutations in genes directly or indirectly affecting GABAergic pathway and the associated epilepsy syndromes, autism and intellectual disability



CAE=Childhood absence
IAE=Idiopathic absence
epilepsy
ID=intellectual disability
LGS=Lennox-Gastaut
syndrome

JME=Juvenile myoclonic
epilepsy
LGS=Lennox-Gastaut
syndrome
MAE=Myoclonic astatic
epilepsy

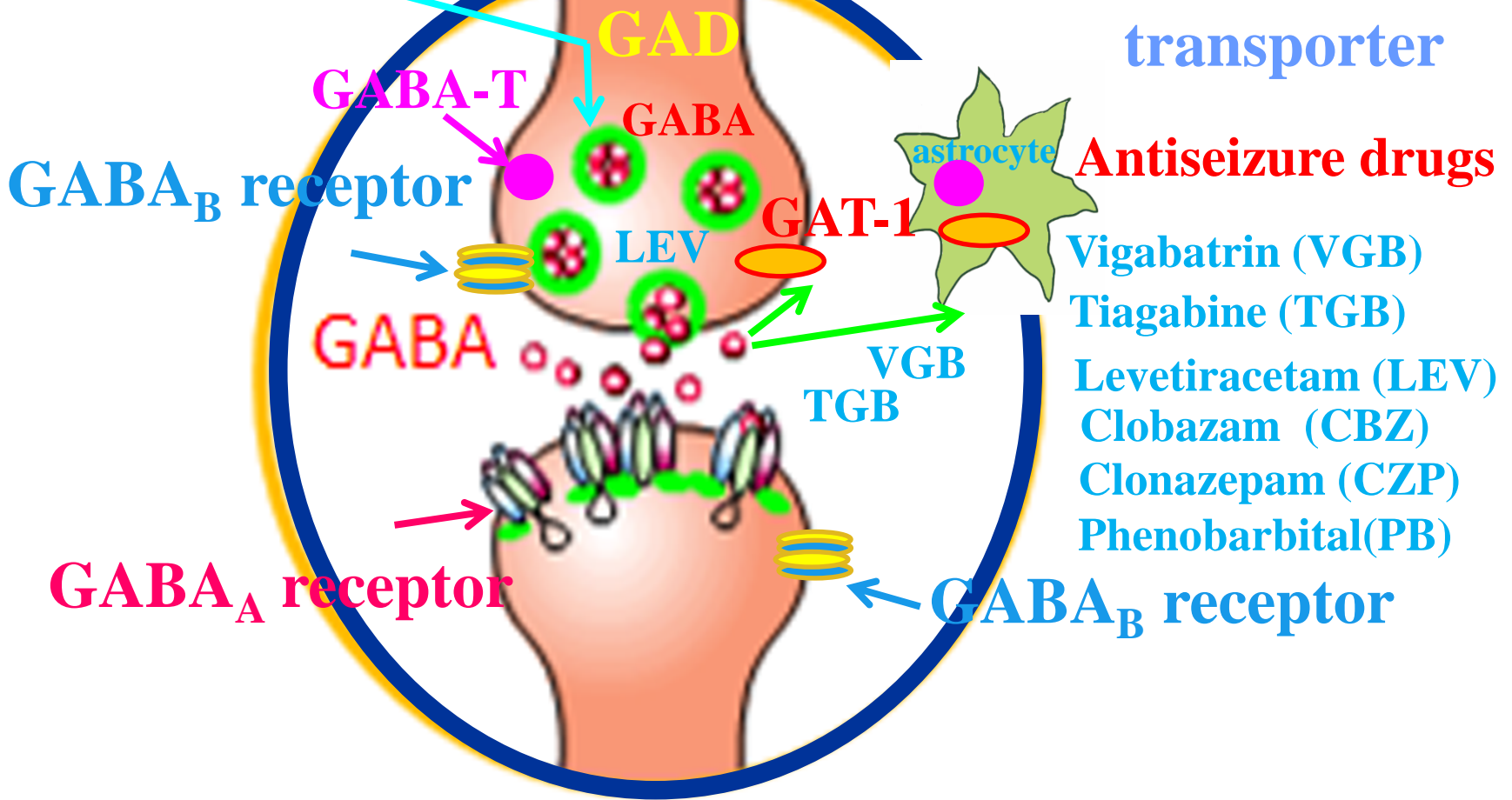
* **Gene will be studied**

GABAergic neurotransmission is a major pathway for epilepsy

GABAergic interneuron

Receptor,
neurotransmitter
transporter

Glutamic acid



Antiseizure drugs

Vigabatrin (VGB)

Tiagabine (TGB)

Levetiracetam (LEV)

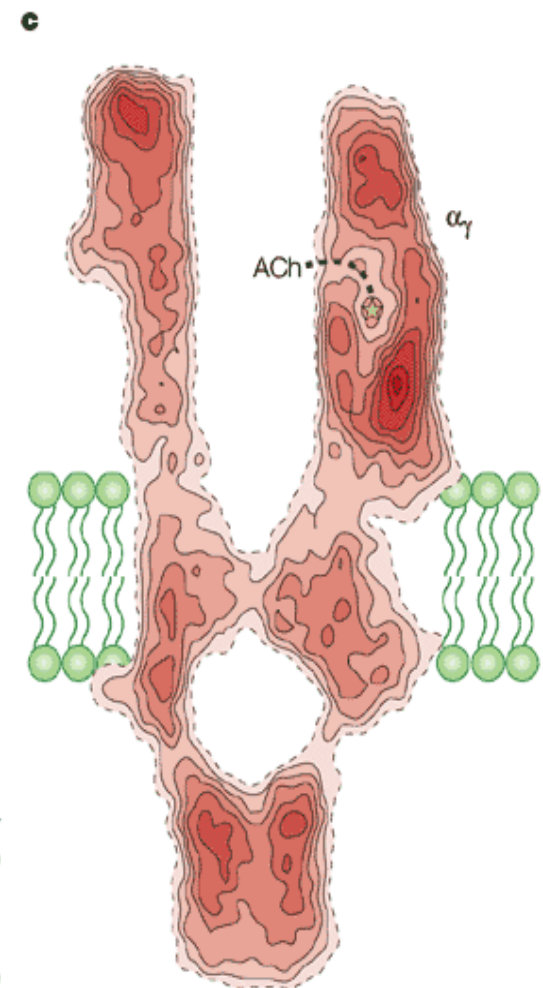
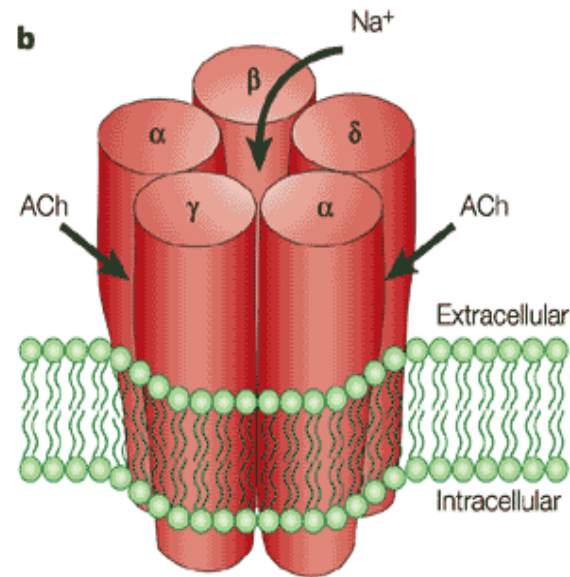
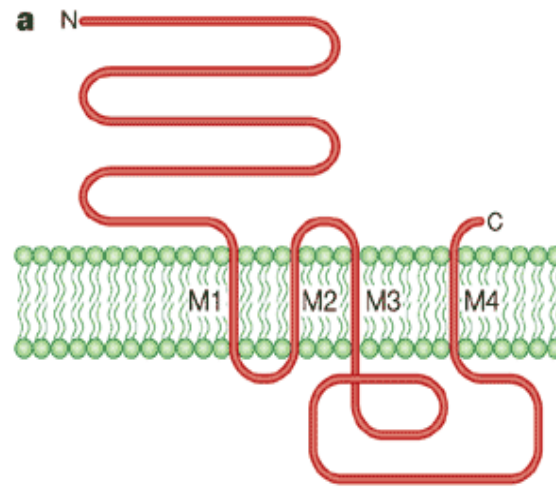
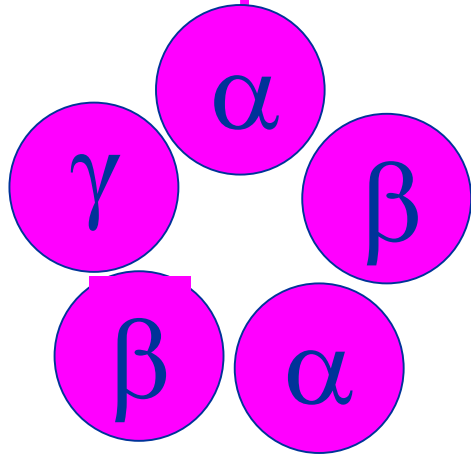
Clobazam (CBZ)

Clonazepam (CZP)

Phenobarbital (PB)

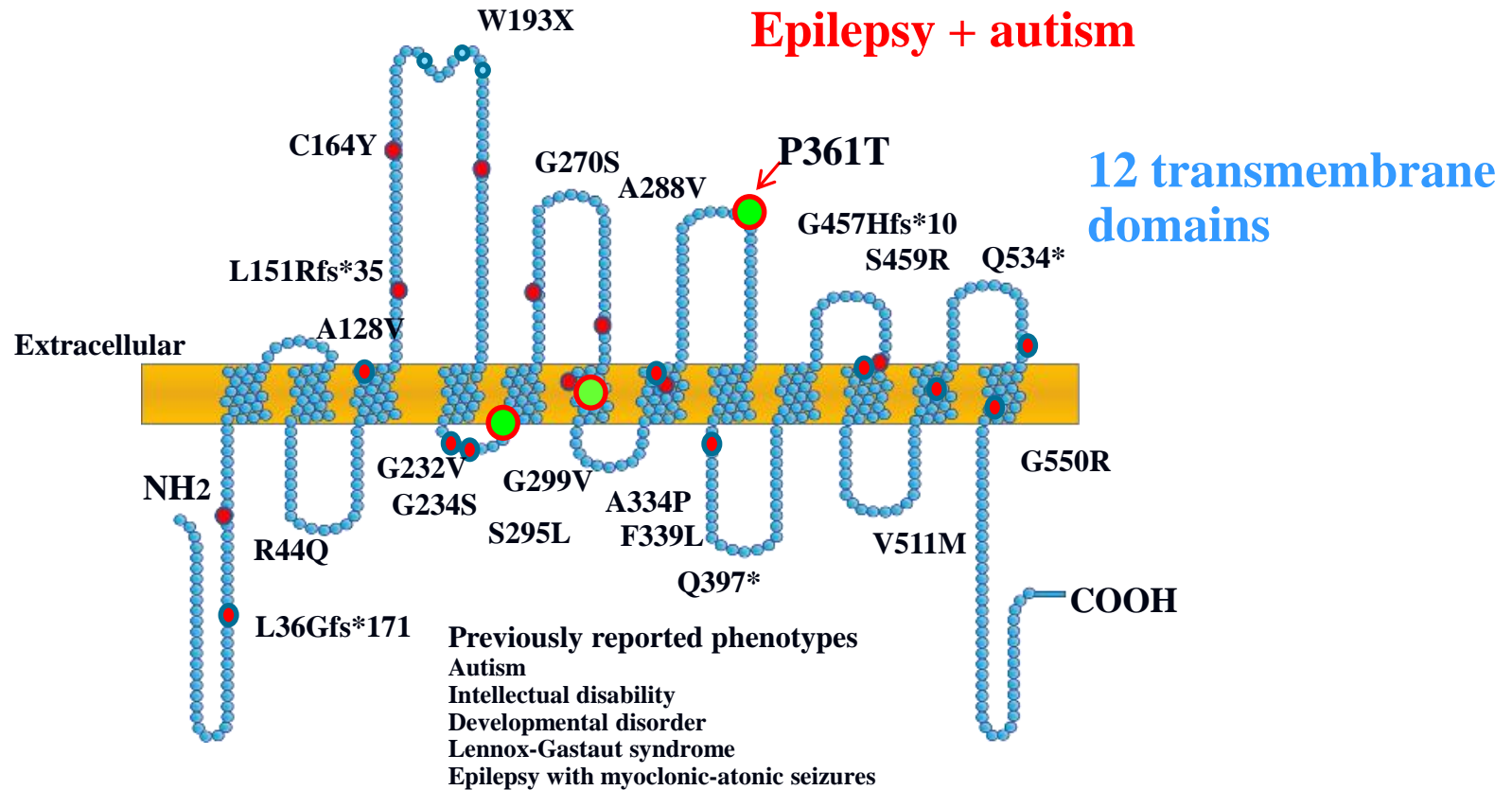
Post-synaptic neuron

Our previous work has mainly focused on GABA_A receptor
We identified GABA transporter shares a lot of features with an
ion channel protein

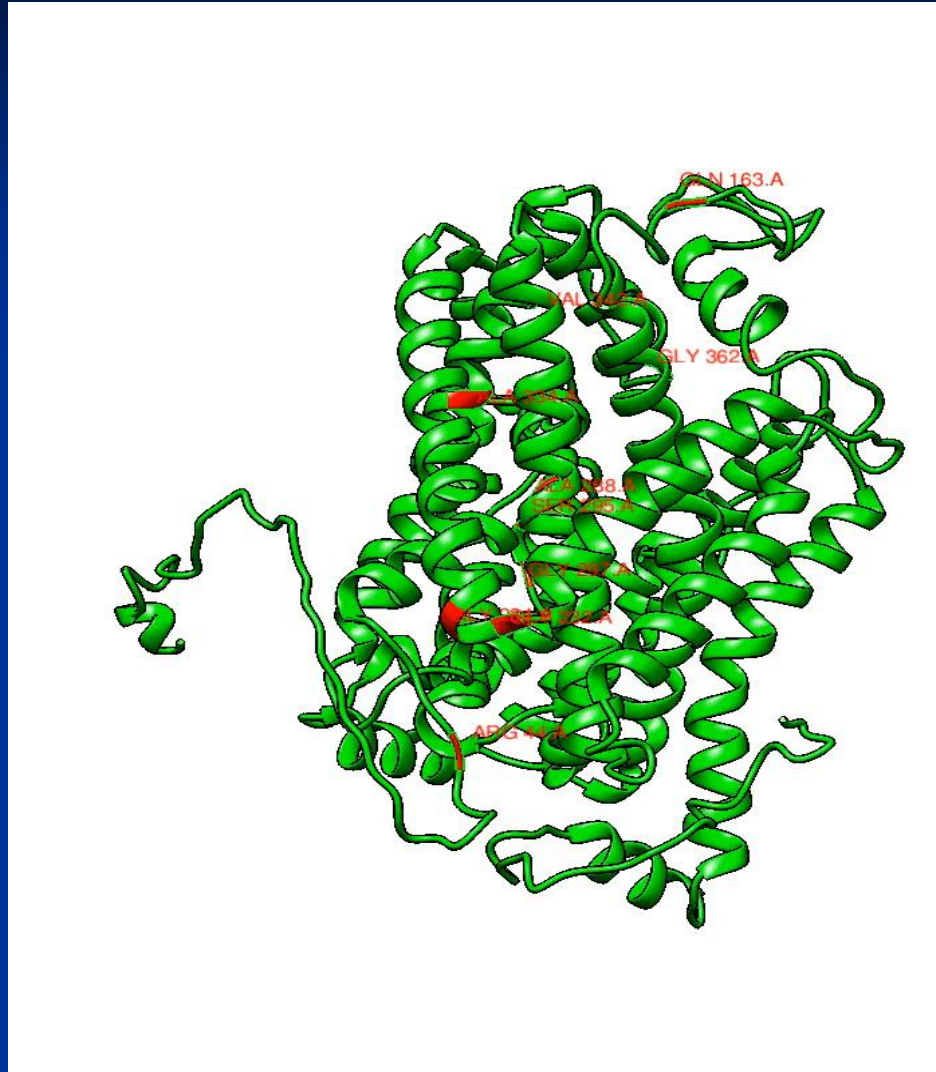


Mutations identified in SLC6A1

**Phenotypes of
P361T:
Epilepsy + autism**



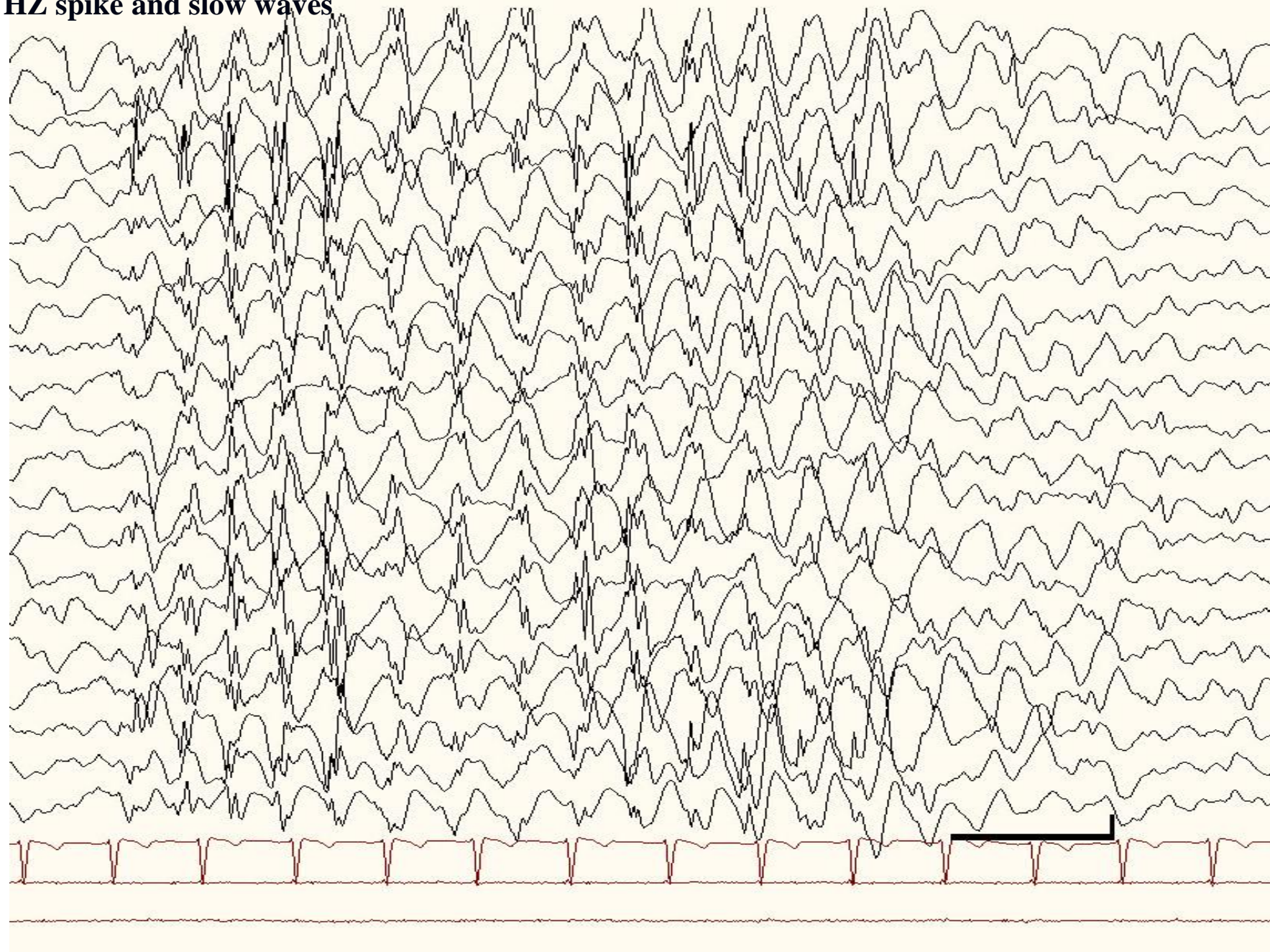
Video showing multiple mutations in GAT-1 associated with epilepsy, autism and intellectual disability



SLC6A1(P361T) associated with autism and epilepsy in a 6-year-old girl, bilateral prefrontal

2.0-3.0 HZ spike and slow waves

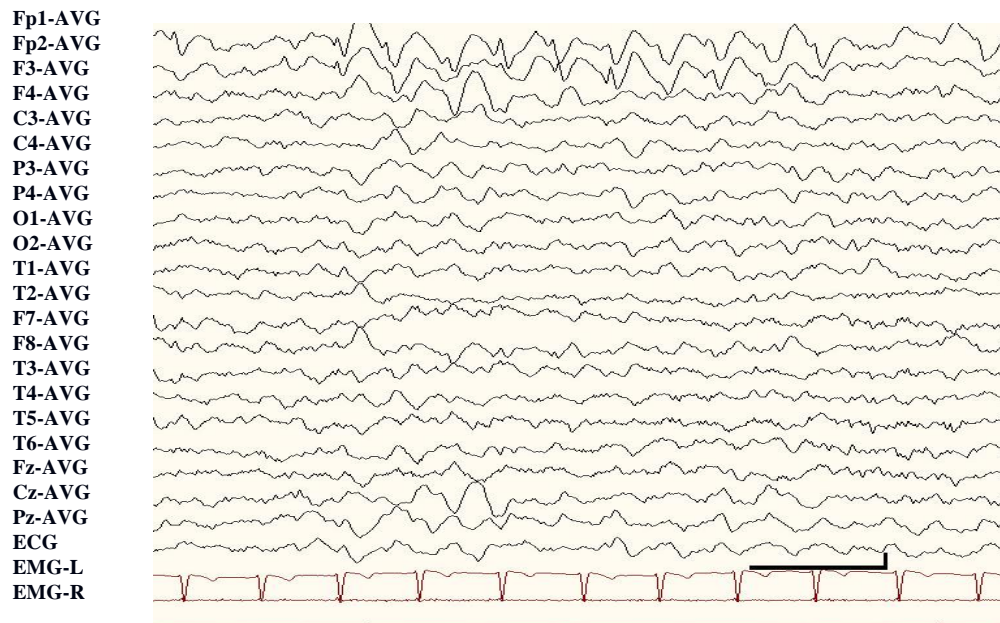
Fp1-AVG
Fp2-AVG
F3-AVG
F4-AVG
C3-AVG
C4-AVG
P3-AVG
P4-AVG
O1-AVG
O2-AVG
T1-AVG
T2-AVG
F7-AVG
F8-AVG
T3-AVG
T4-AVG
T5-AVG
T6-AVG
Fz-AVG
Cz-AVG
Pz-AVG
ECG
EMG-L
EMG-R



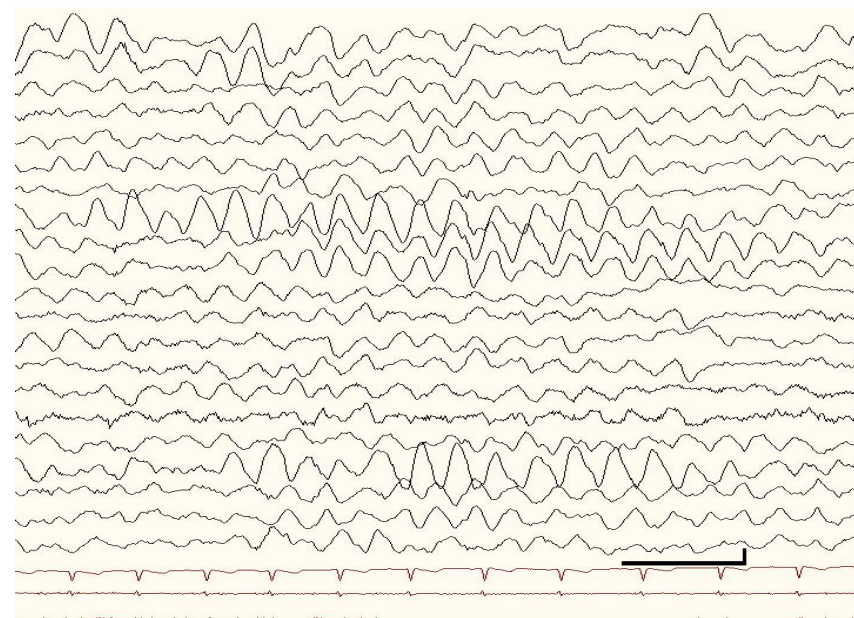
Fp1-AVG
Fp2-AVG
F3-AVG
F4-AVG
C3-AVG
C4-AVG
P3-AVG
P4-AVG
O1-AVG
O2-AVG
T1-AVG
T2-AVG
F7-AVG
F8-AVG
T3-AVG
T4-AVG
T5-AVG
T6-AVG
Fz-AVG
Cz-AVG
Pz-AVG
ECG
EMG-L
EMG-R

Bilateral multifocal 2.0-3 HZ spike and slow wave discharges

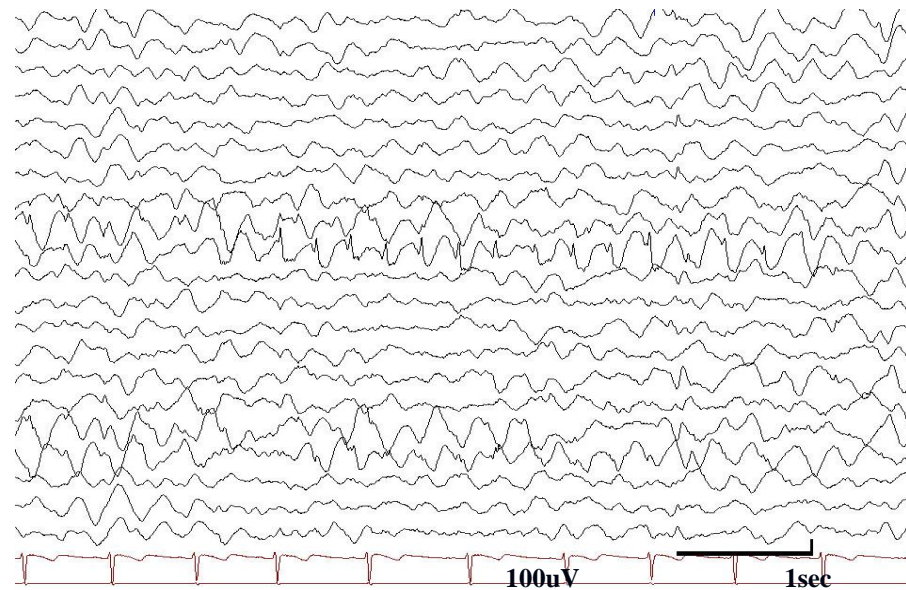
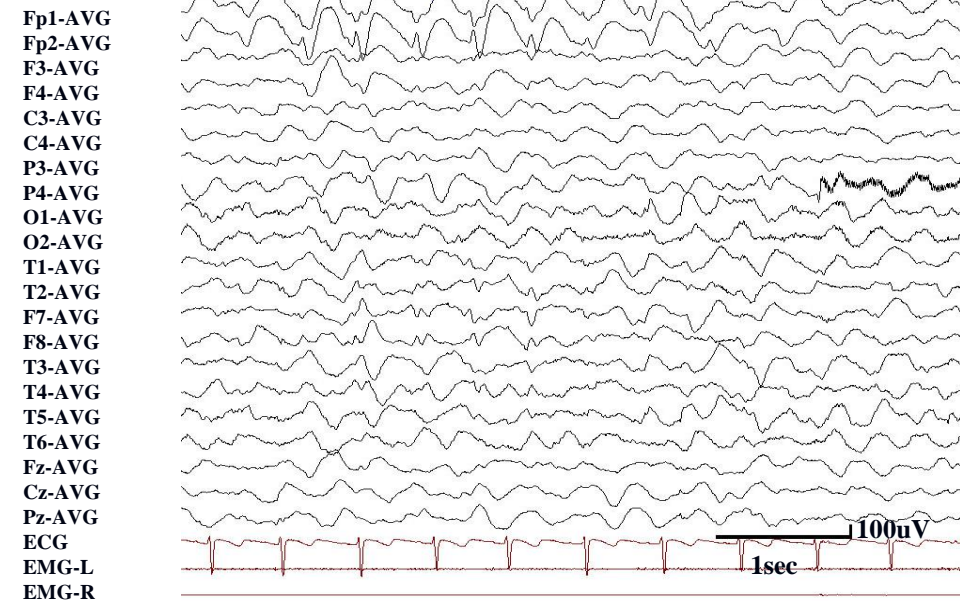
A



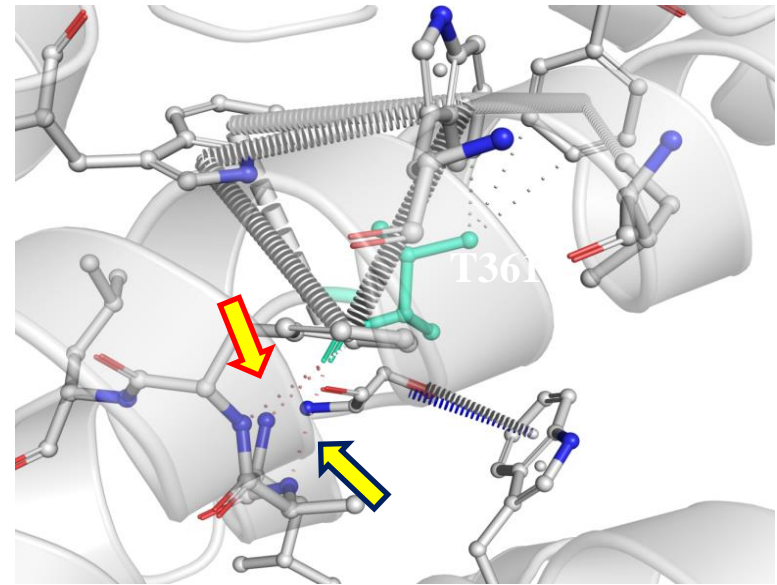
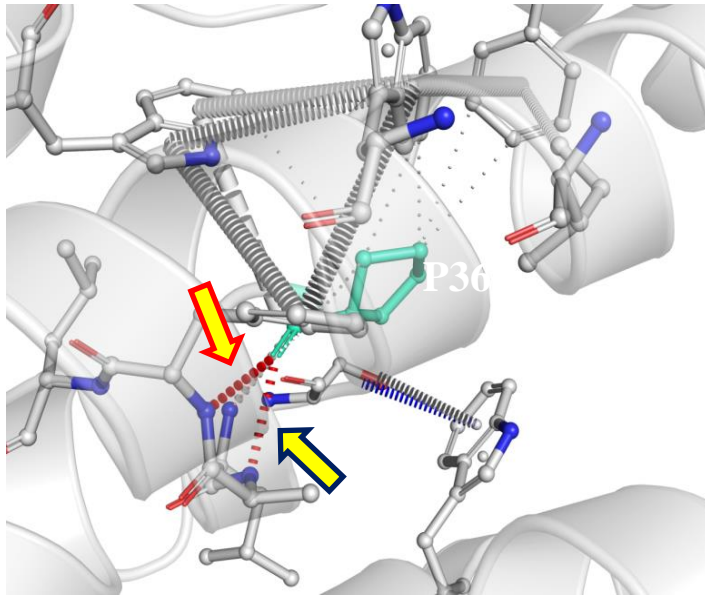
B



D

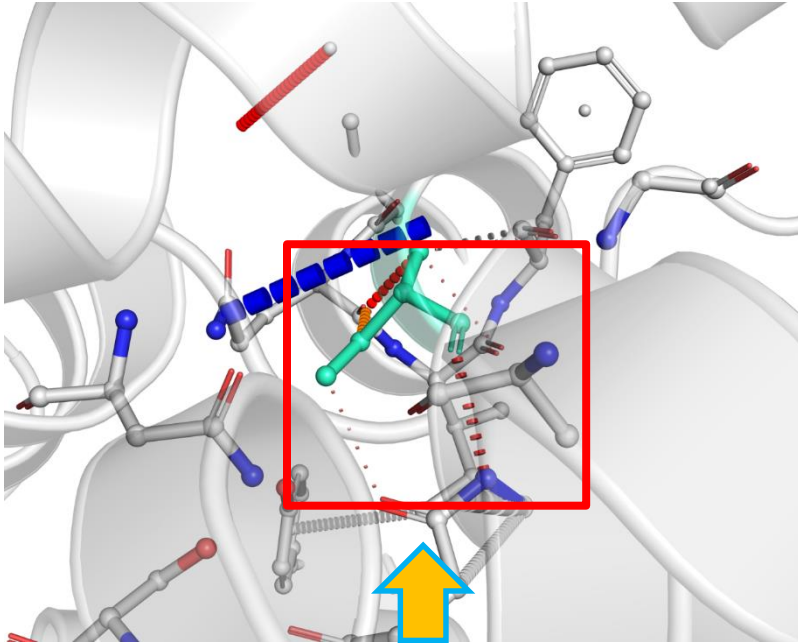


Machine learning and structure modeling showing loss of a hydrogen bond, resulting in a destabilized mutant protein

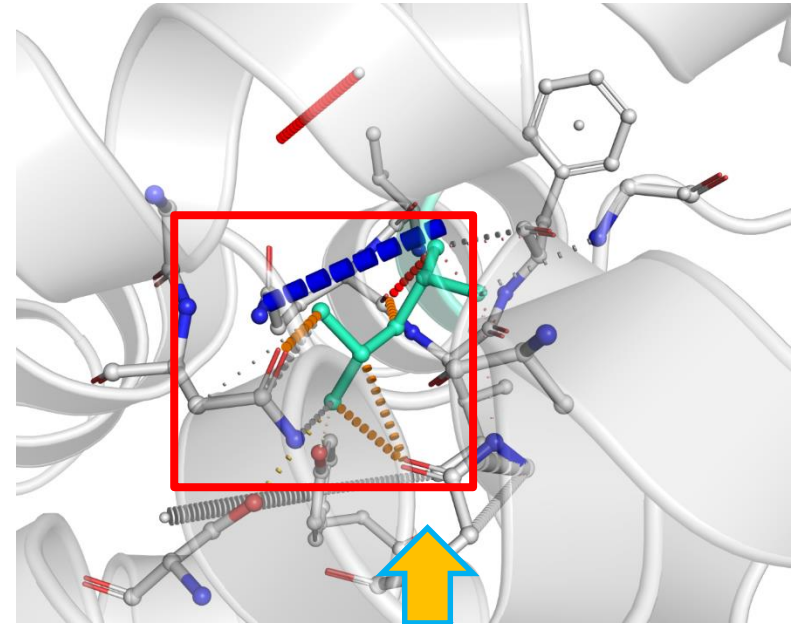


Machine learning and structure modeling showing form of more hydrogen bonds.

Wildtype GAT-1(S295)



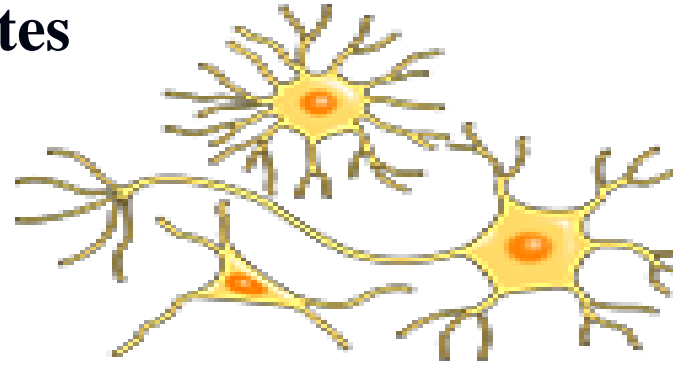
mutant GAT-1(L295)



Mutation from Serine to Leucine may bring huge impact. The side chain polarity is mutated from polar to nonpolar, and Serine is hydrophilic but Leucine is hydrophobic. According to the images, more hydrogen bonds formed.

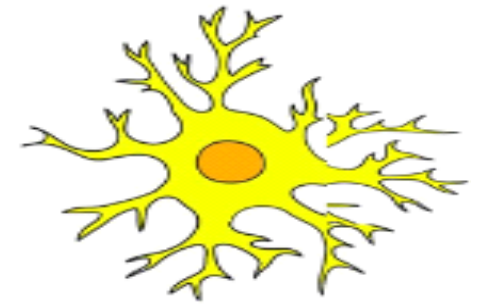
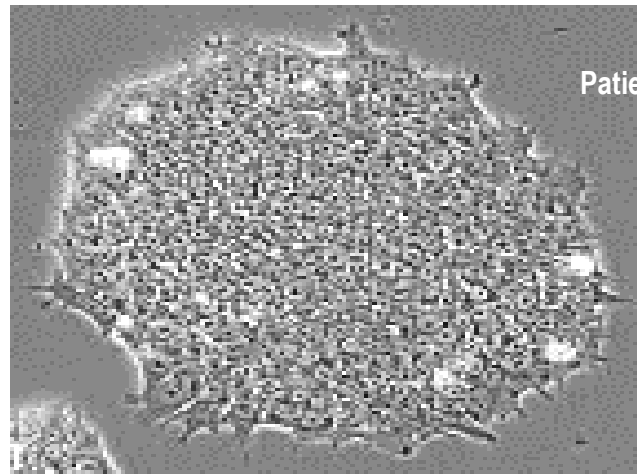
How to study the impact of a mutation?

We have studied the traffick and function of mutant GAT-1 in HEK293T, iPSCs, mouse neurons and astrocytes



Nerve cells

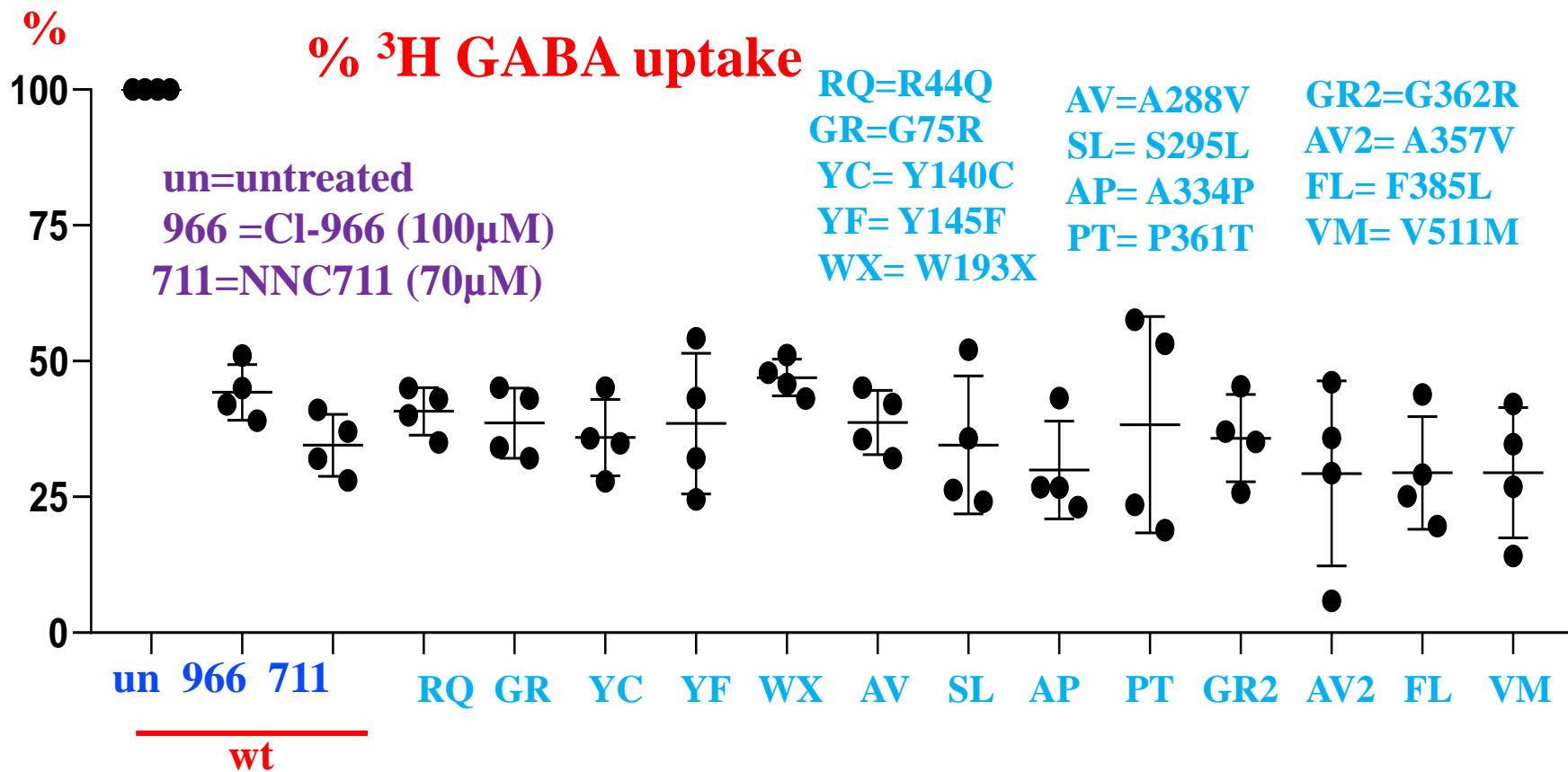
iPSCs



astrocytes

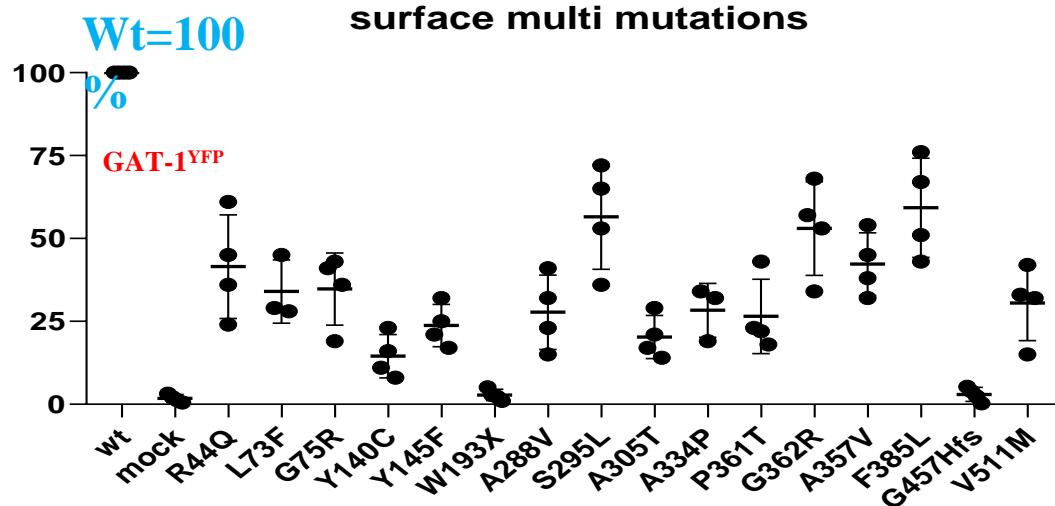
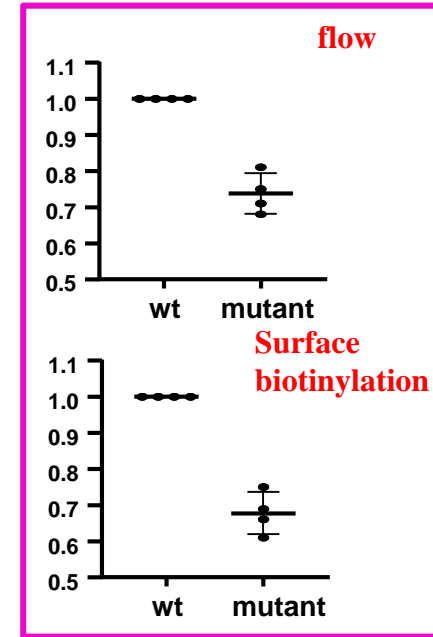
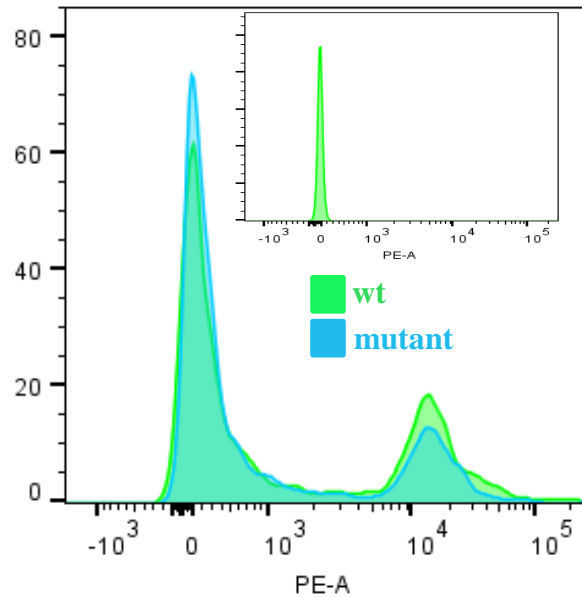
What is the function of the mutant transporters?

Neuron



Can mutant GAT-1 traffick to cell surface?

Using a high-throughput assay



Phenotypes

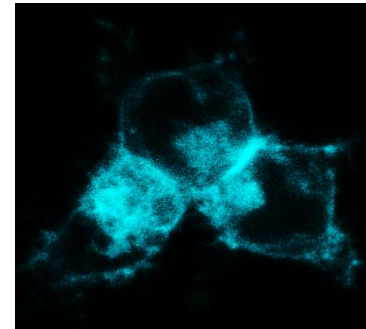
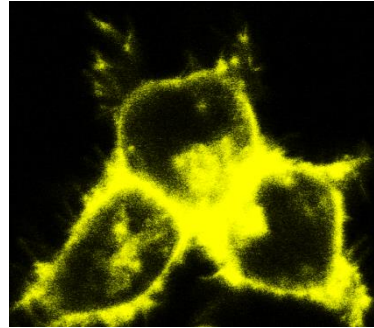
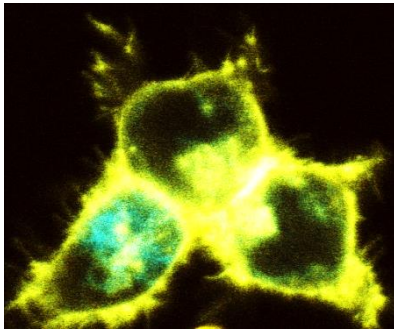
LGS, MAE, CAE, Autism, ID

overlay

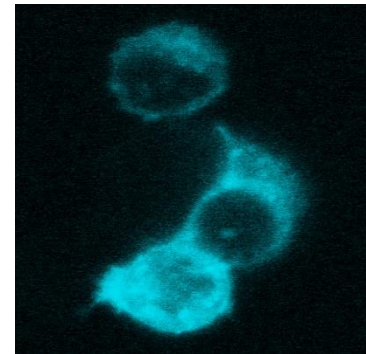
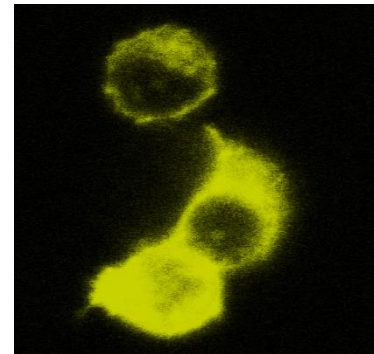
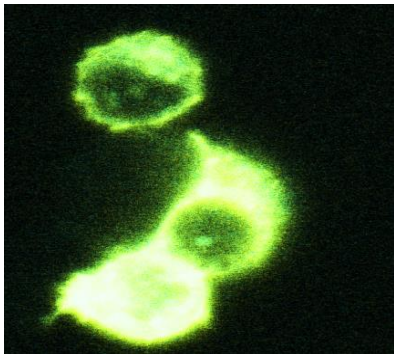
GAT-1^{YFP}

ER^{CFP}

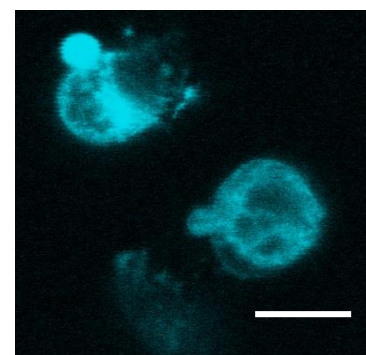
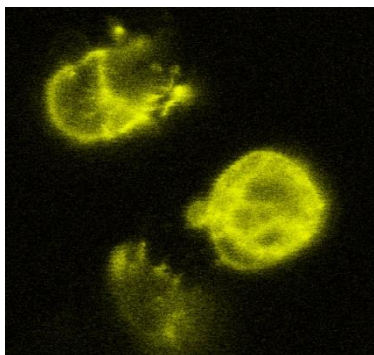
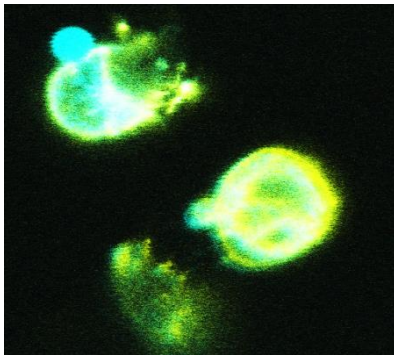
wt



P361T

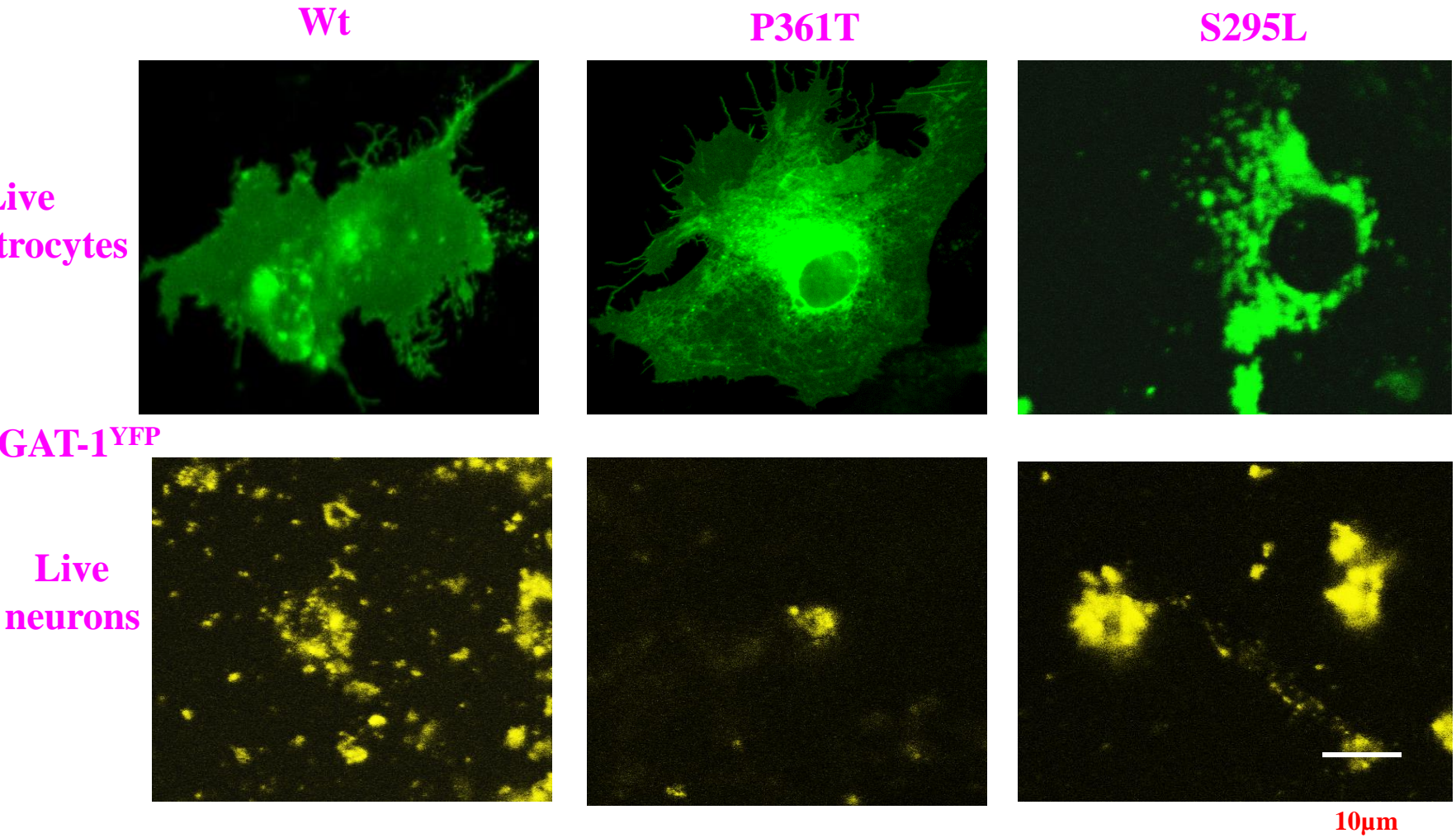


+Tuni



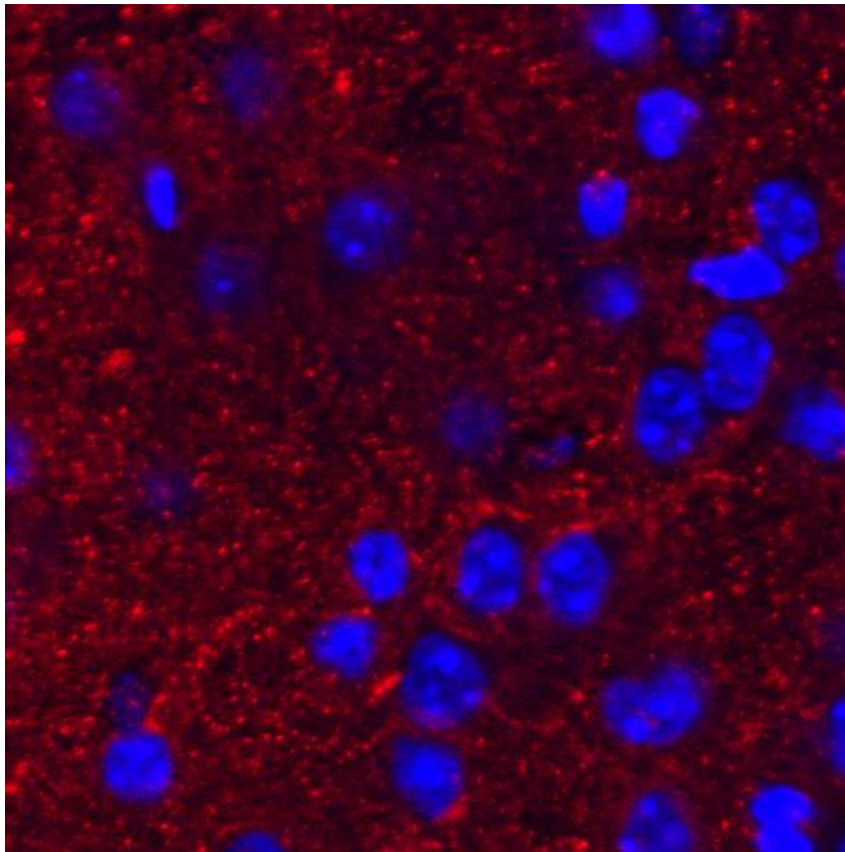
10μm

astrocytes could be a good tool for GAT-1 trafficking study

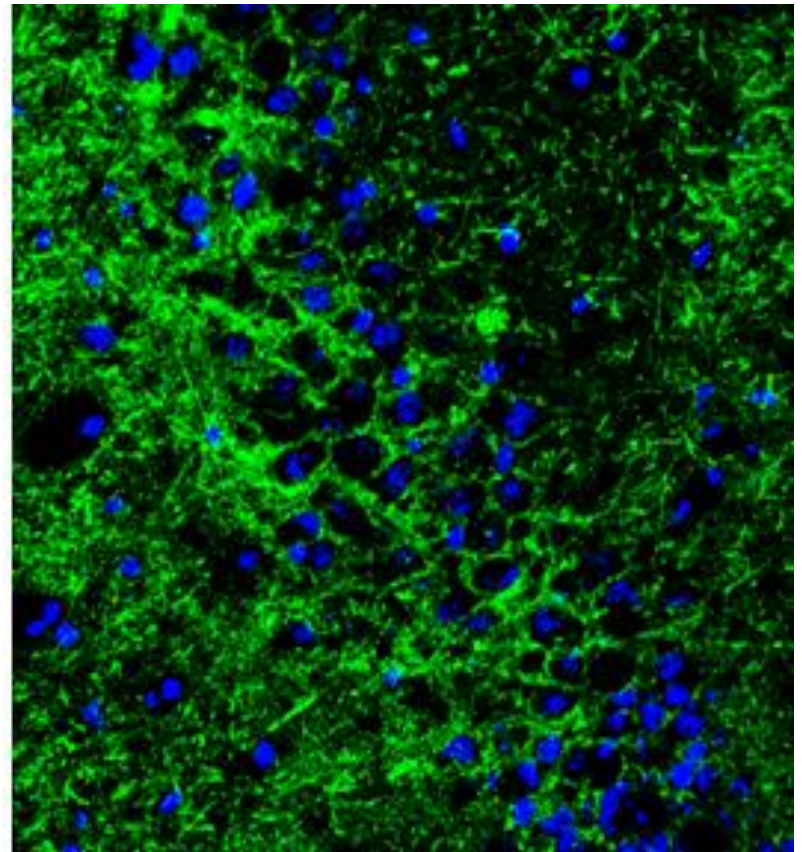


**GAT-1 immunoreactivity in cortex (red) and hippocampus
(green)**

3 months old mouse in C57/BL/6J mouse background



Cortex



Hippocampus

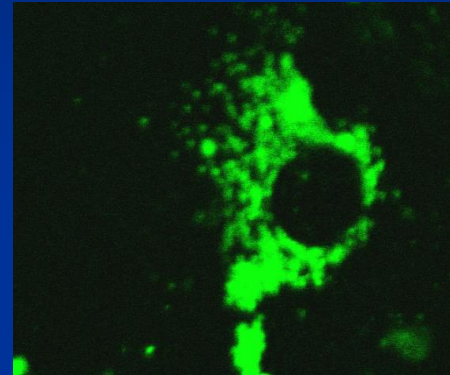
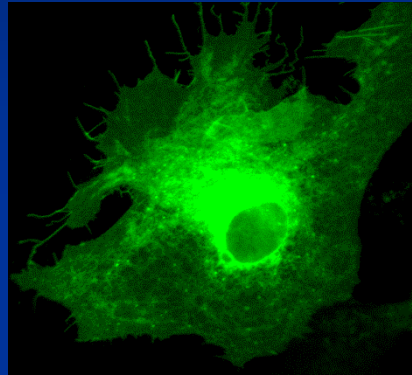
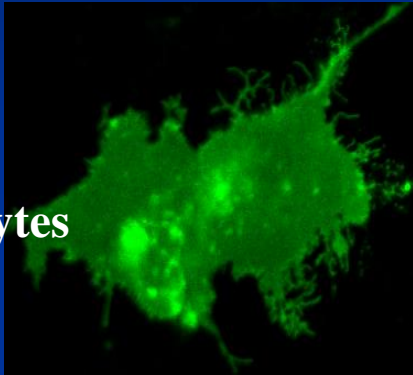
Mouse tissue

Wt

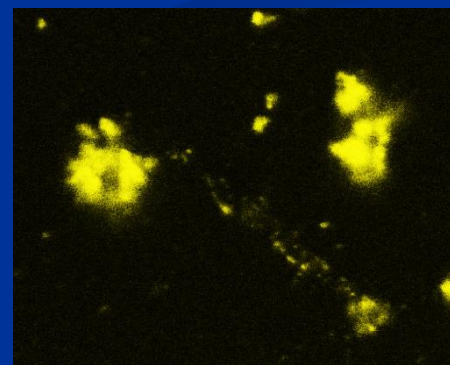
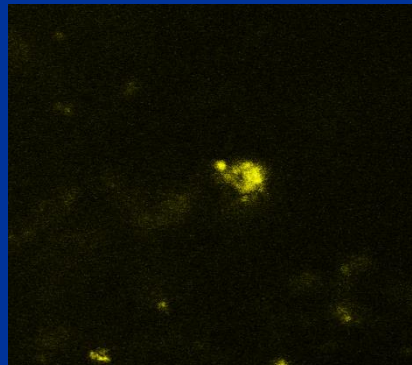
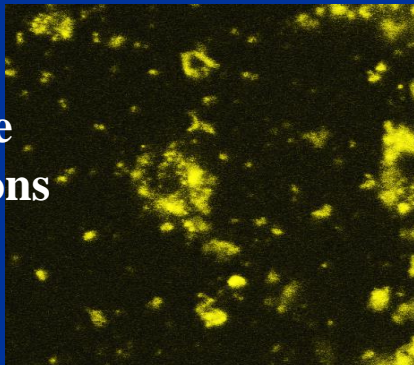
P361T

S295L

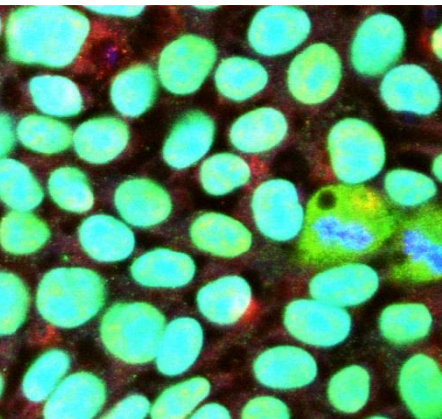
Live
Astrocytes



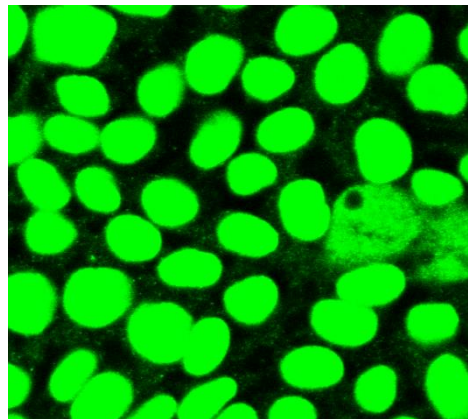
Live
neurons



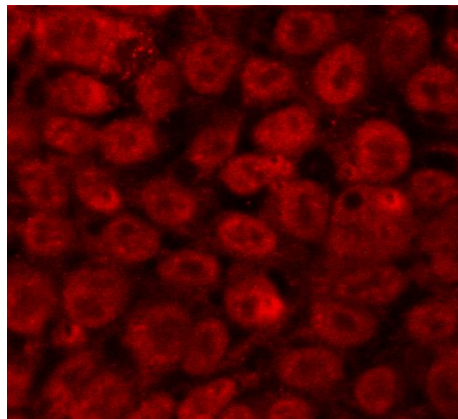
overlay



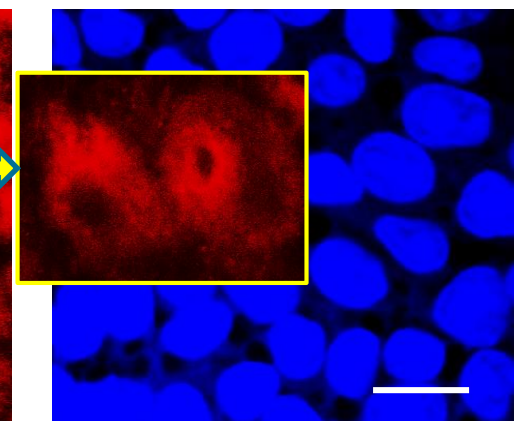
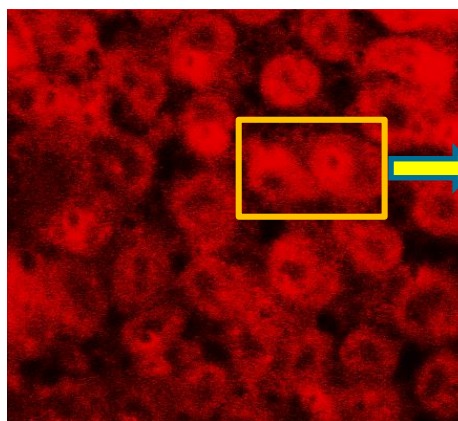
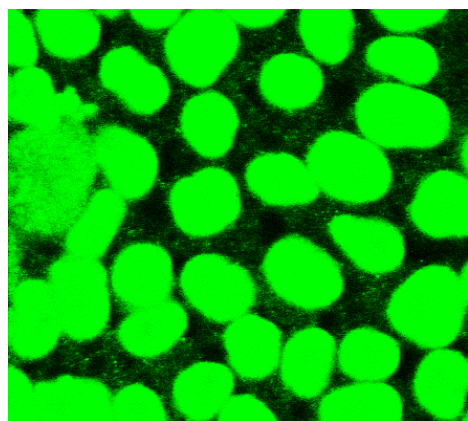
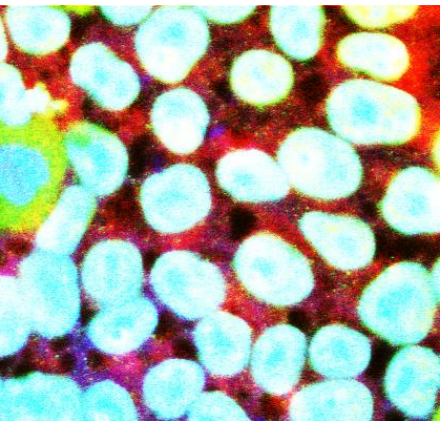
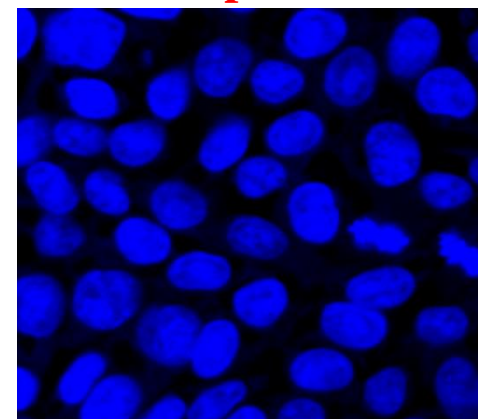
oct4



GAT-1



To-pro-3



10μm

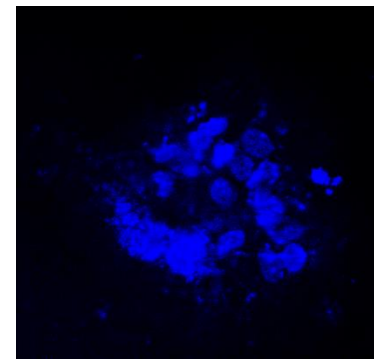
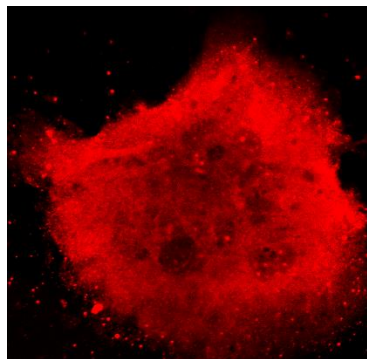
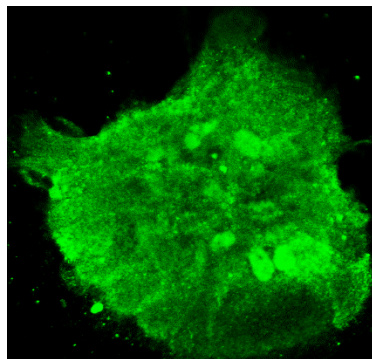
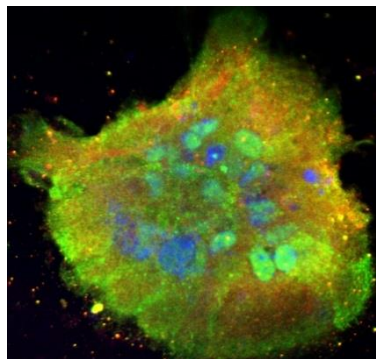
overlay

GAT-1^{YFP}

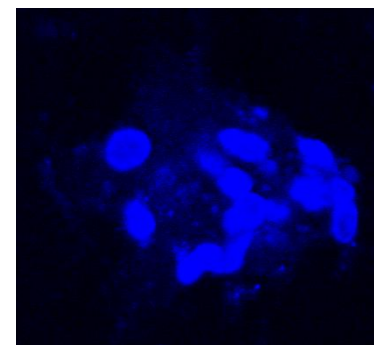
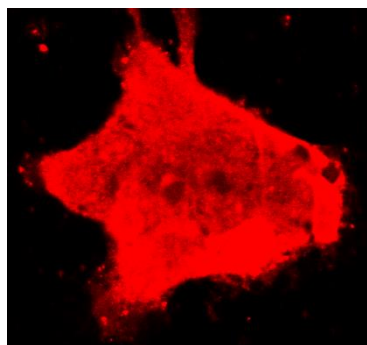
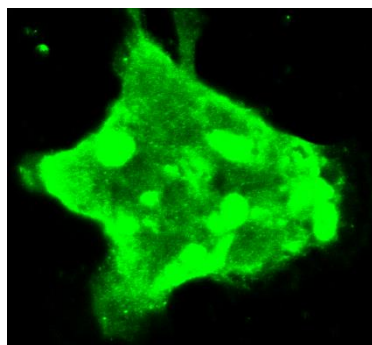
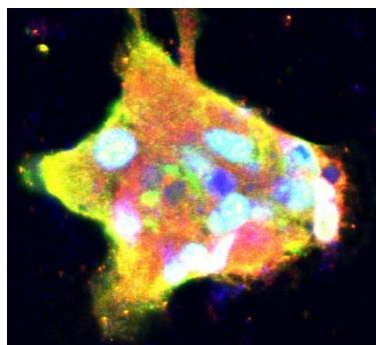
GAT-1

To-pro-3

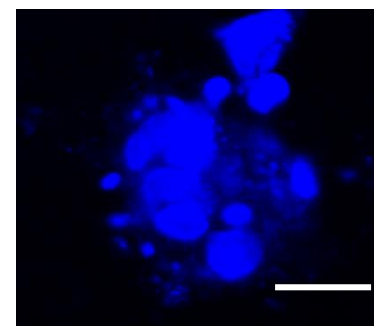
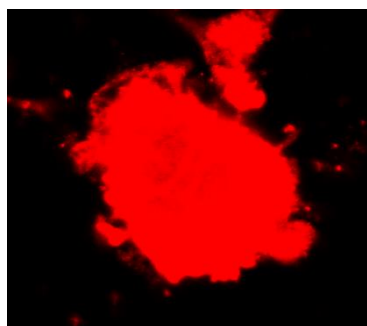
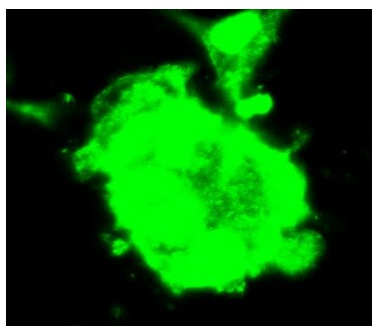
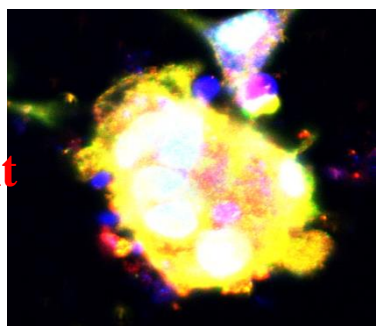
wt



mutant



mutant



10μm

Will GAT-1 form dimers?

1 2 3

HeLa cells

³⁵S

Dimer ?

1. Immunoprecipitation with the affinity-purified antibody against GABA transporter monomer

2. Immunoprecipitation with anti-GABA transporter antiserum

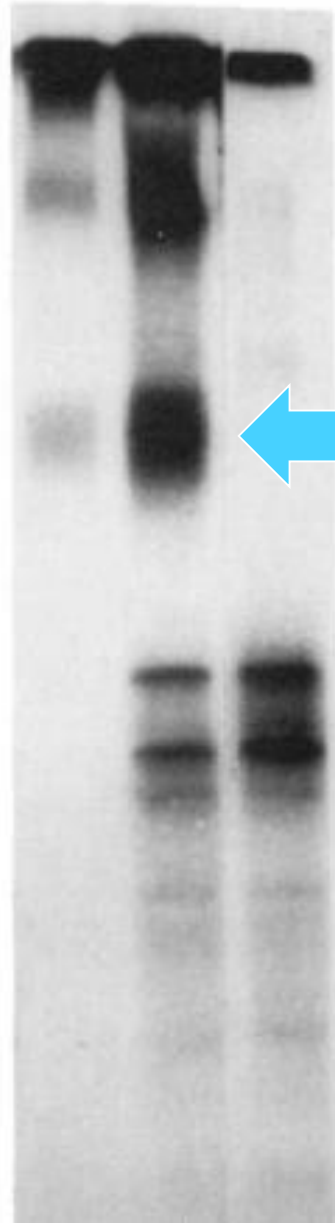
67kDa

43kDa

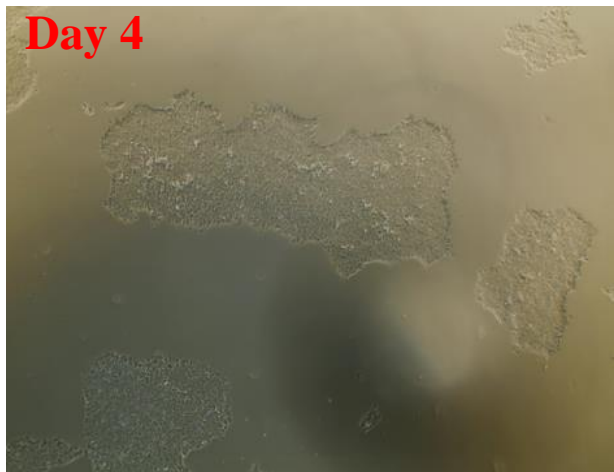
30kDa

20.1kDa

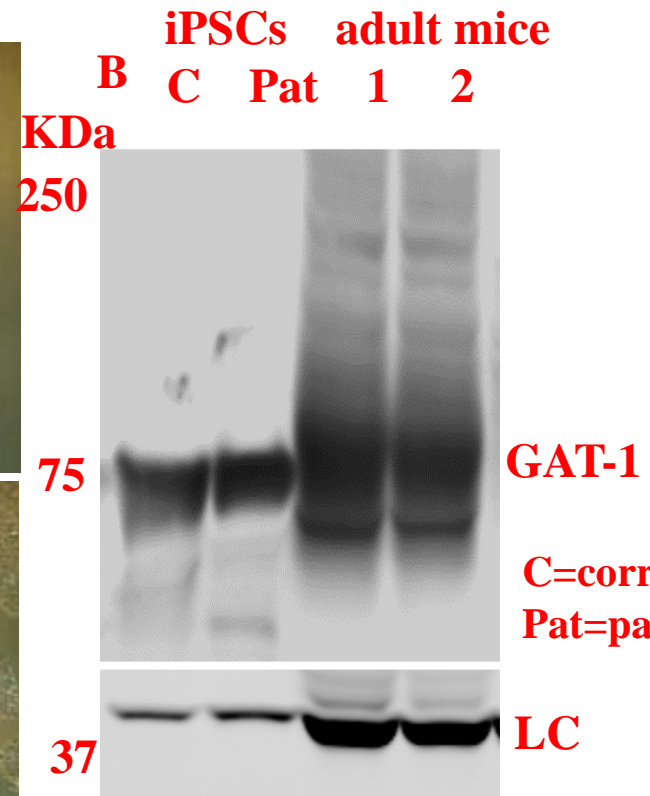
14.4kDa



A 10X corrected iPSCs

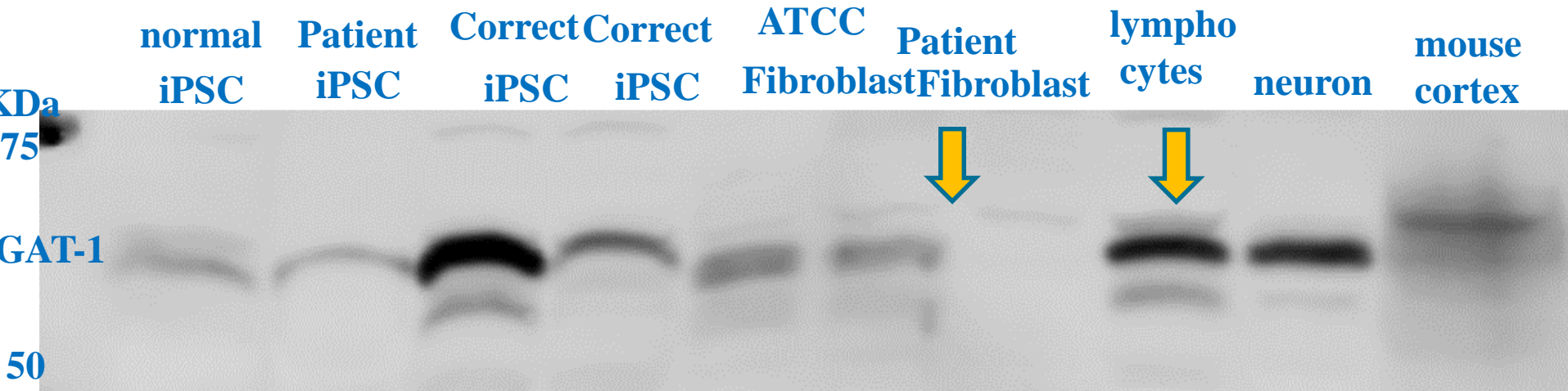


iPSCs patient

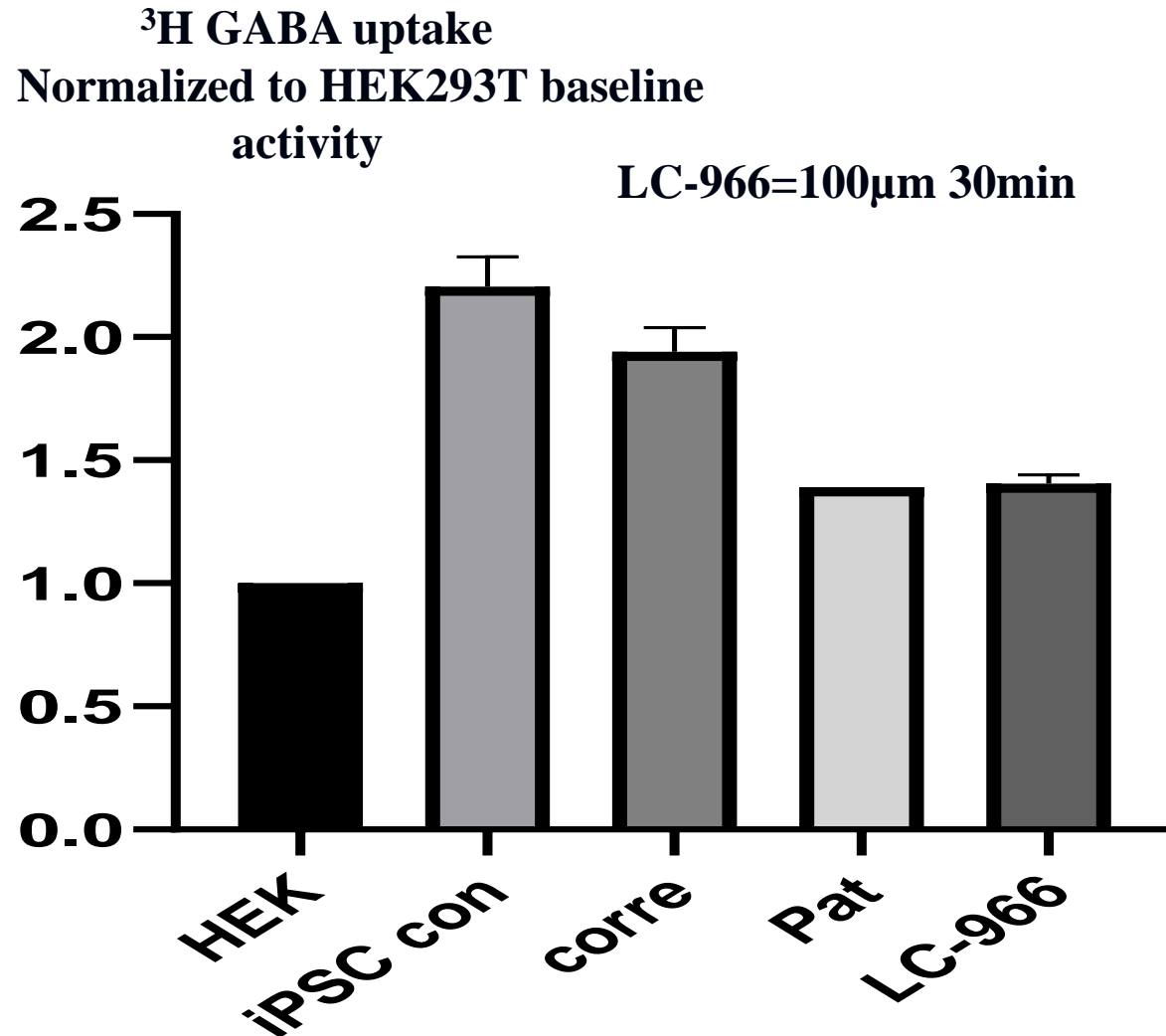


1. GAT-1 is expressed in iPSCs, fibroblasts and lymphocytes

2. GAT-1 in stem cells and mature neurons may have different posttranslational modifications



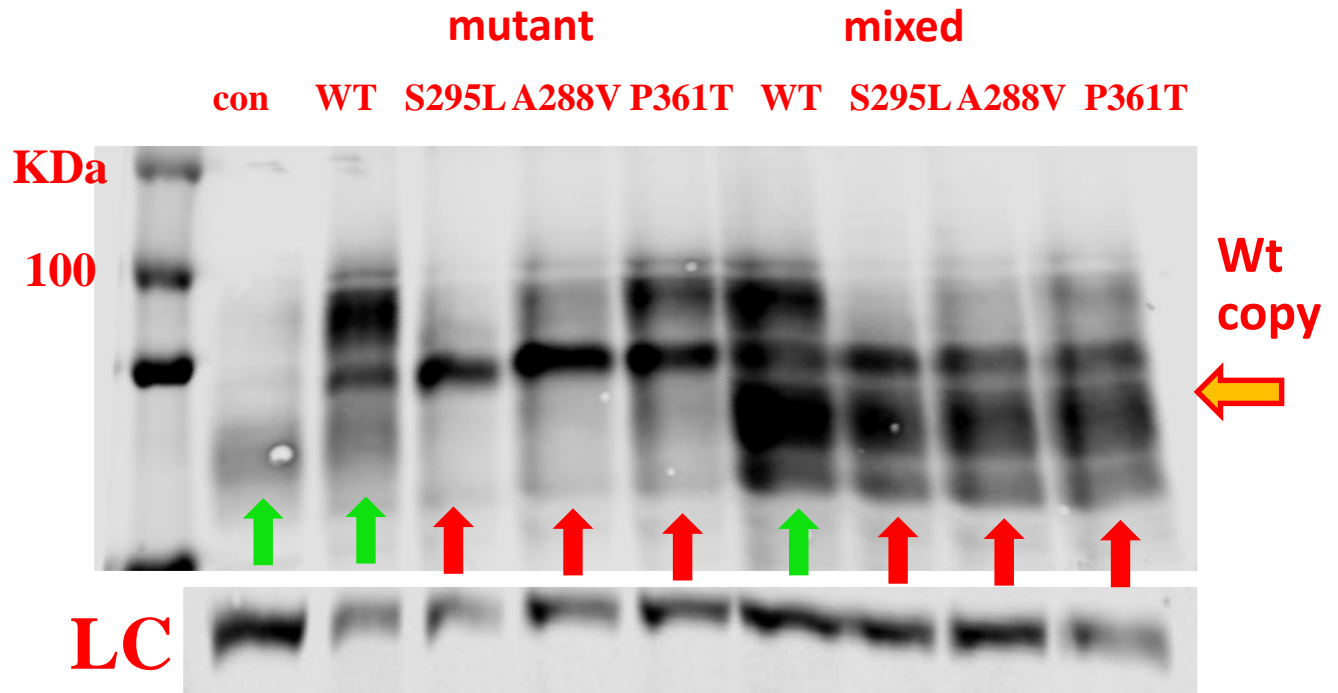
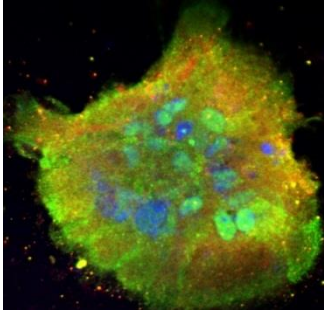
Human iPSCs only had minimal GABA uptake activity



Astrocytes

Mutant GAT-1 interfered the expression of the endogenous GAT-1

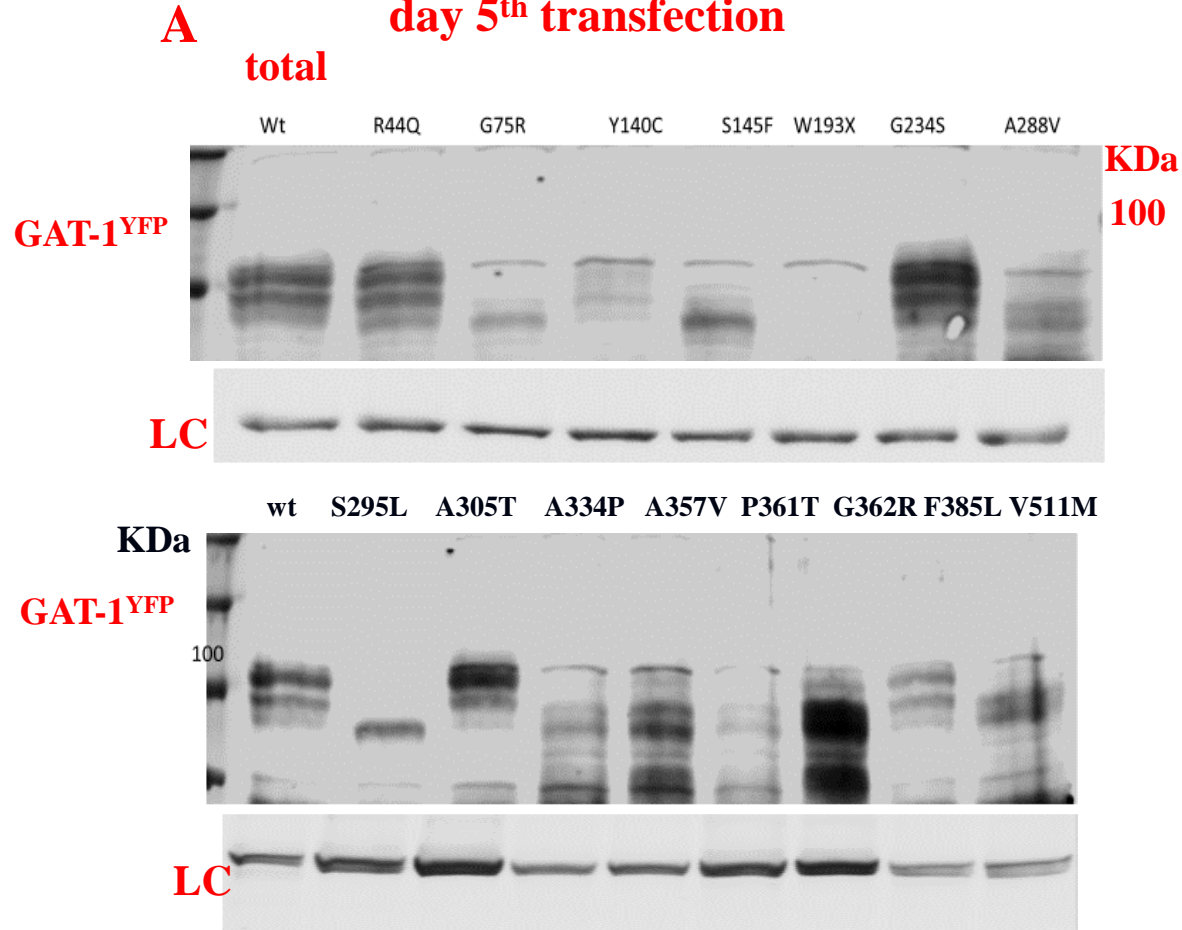
GAT-1 GABA uptake activity was measured at day 5 (P0) in dish.



Mutant GAT-1 had diverse expression pattern in mouse neurons

Neurons

17 days old mouse cortical neurons
day 5th transfection



Slc6a1^{+/*A288V*} mutant pups have better survival rate than *Gabrb3*^{+/*N328D*} pups (LGS)

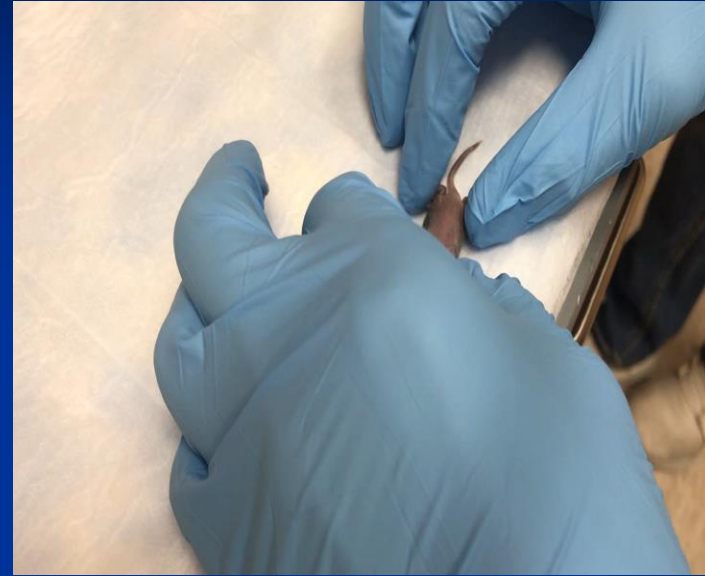
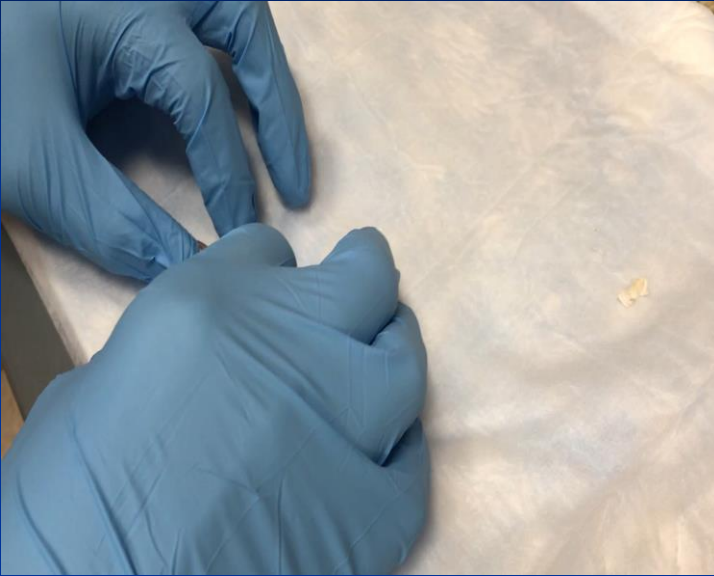
SLC6A1^{+/*A288V*}



Gabrb3^{+/*N328D*}

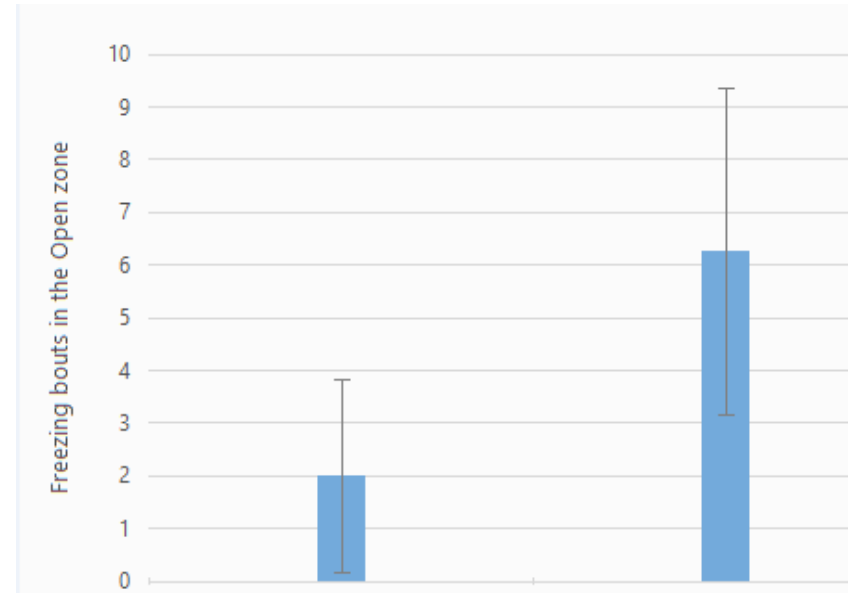
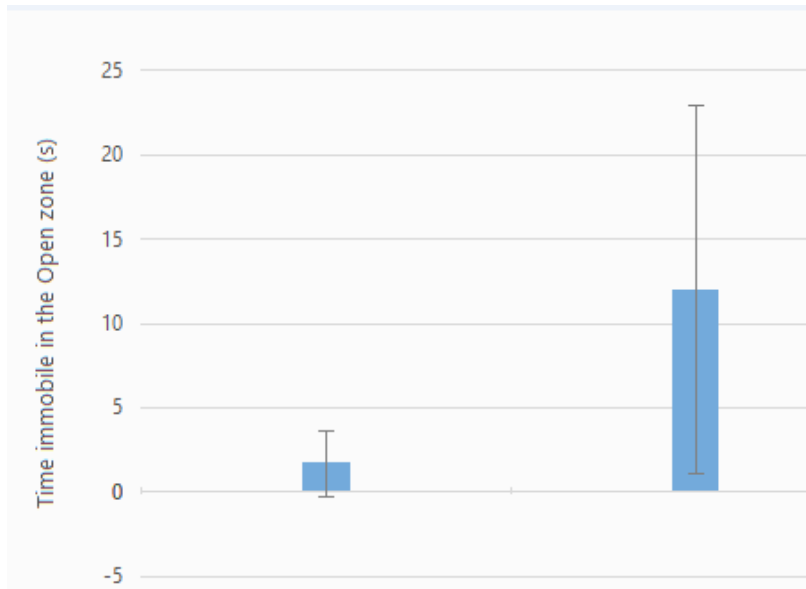


Righting reflex

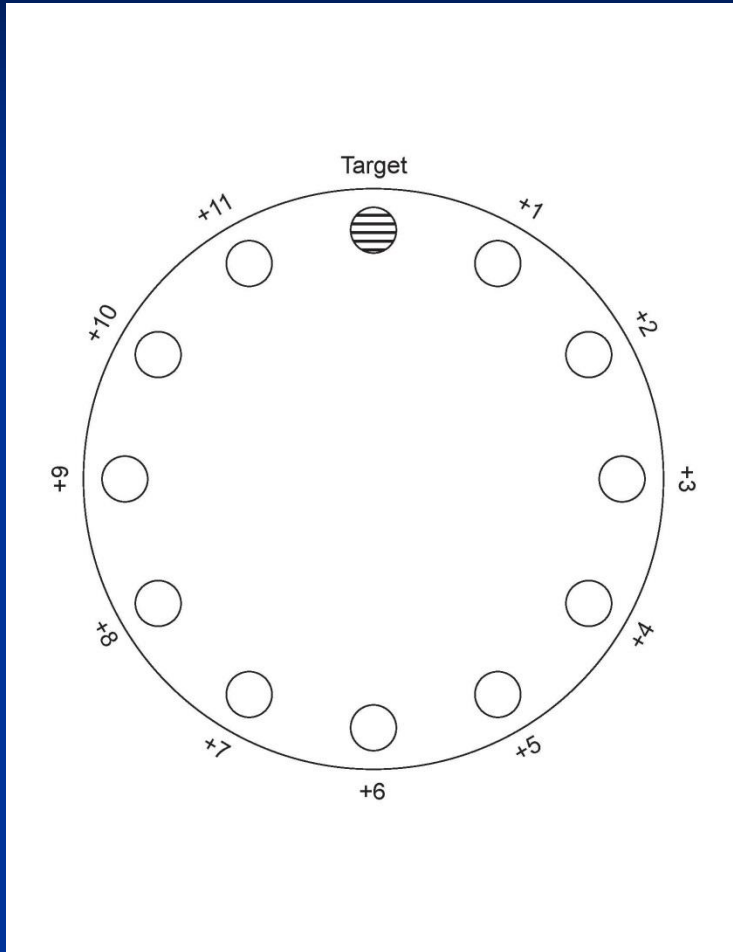


The mutant *Slc6a1*^{+/*A288V*} mice had seizures

Anxiety in the mutant *Slc6a1*^{+/-A288V} mice



Barnes Maze is less stressful than Water Maze and was used for cognition test .



Barnes Maze Overview

Pretraining
Orientation



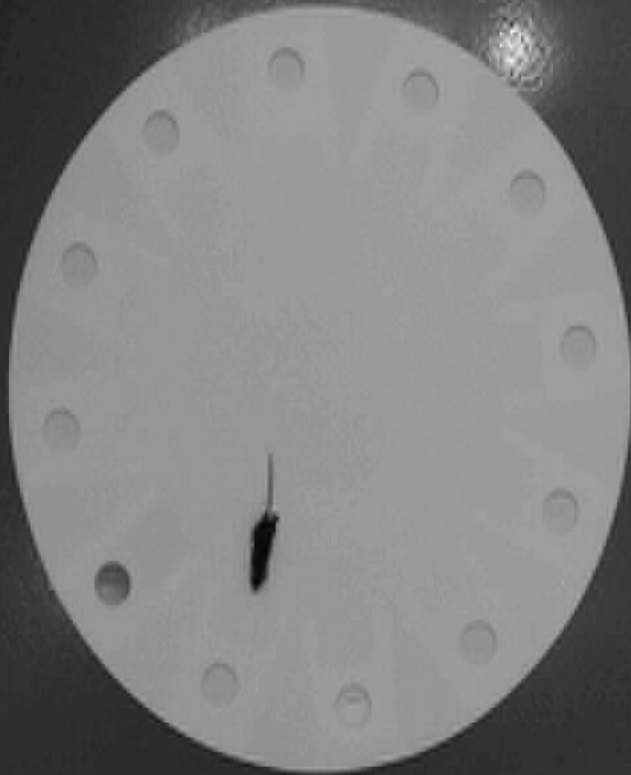
Training
Learning



Probe
Memory

Barnes Maze

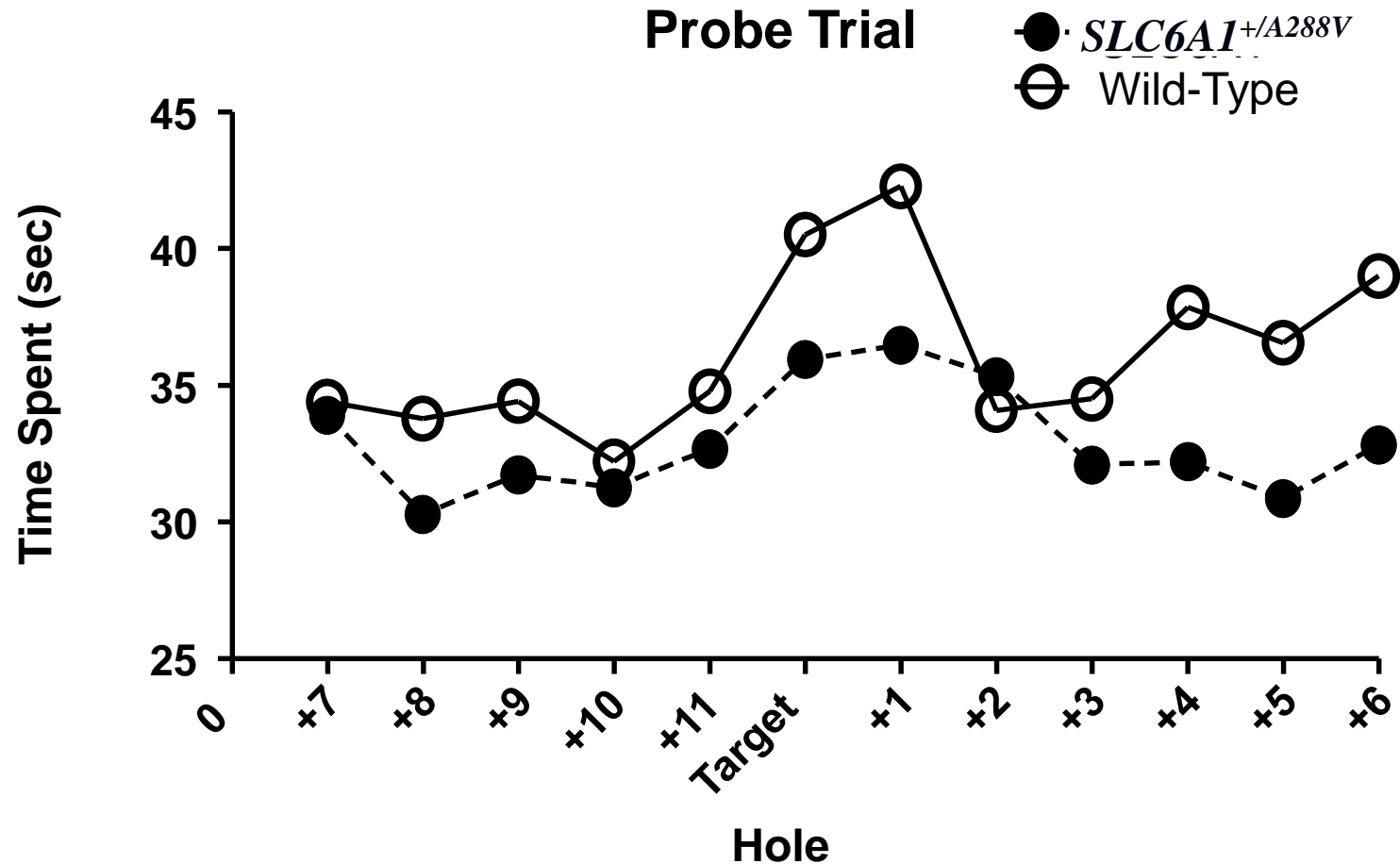
Mutant mouse



Wildtype mouse



Ongoing work with Barnes maze test in the mutant *Slc6a1*^{+/*A288V*} mice



Thanks for the invitation!



Musical city convention center in Nashville

mutant

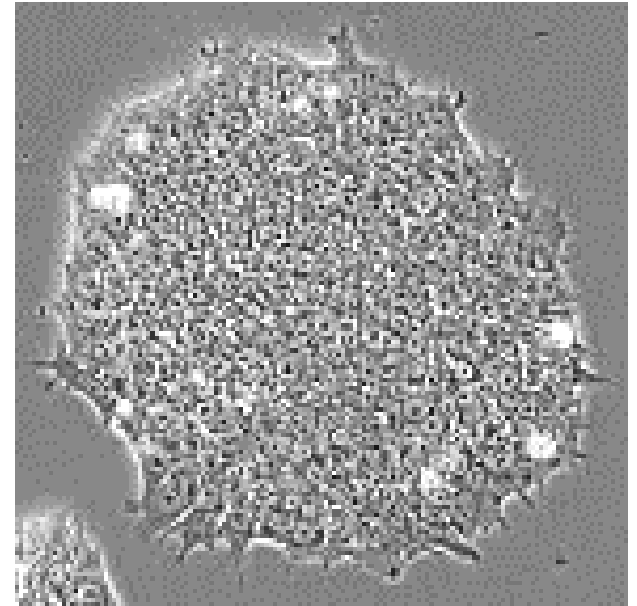


models

mutations



mouse
model

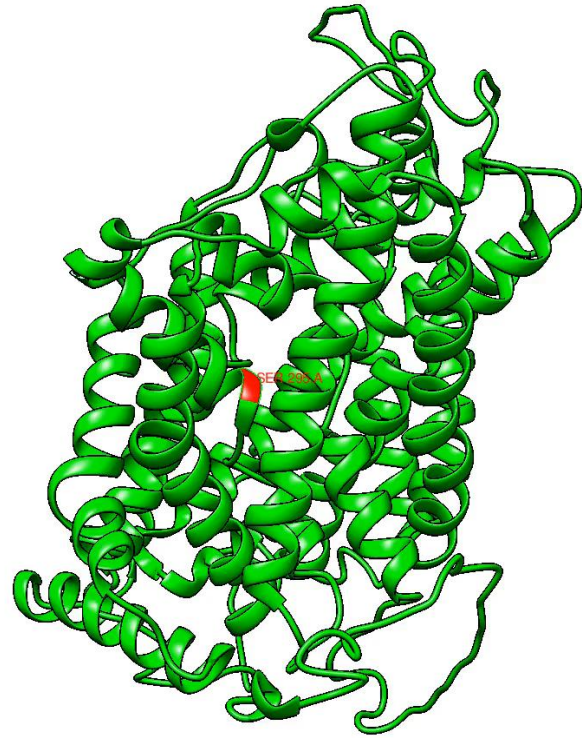


patient
iPSC line

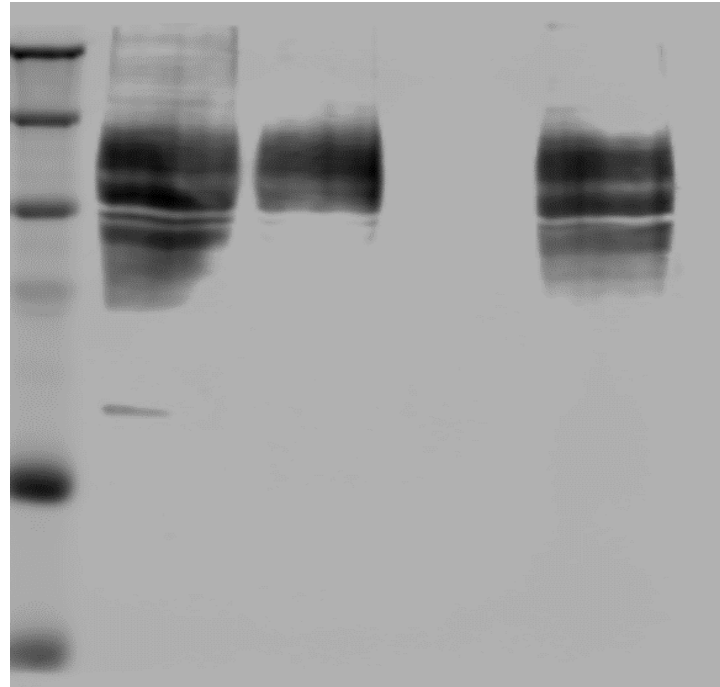




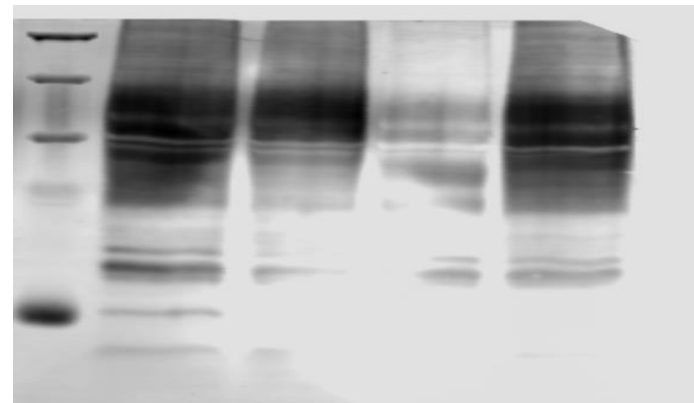
End



Wt R44Q S295L G234S



GAT-1



GAT-1