

Discussion with GABA_A Alliance

Tingwei Mu
Associate Professor
Department of Physiology and Biophysics
Department of Neuroscience
Case Western Reserve University School of Medicine
Cleveland, OH

Justin's Questions

- a) How A1, B2, G2 variants are differing in your experiments? Are some more responsive to certain mechanisms of treatment?
- b) Is there a way to group variants together within each subunit as responding to method # 1, 2, 3, or a combination. Especially if this can be visualized in a chart.
- c) Gain vs. loss of function relating to your experiments. Many of us speak to Dr. Moller's team regularly and we are trained to think in this terminology.
- d) Is the expectation that single agents may modify the disease or is the focus mostly on working toward combinations?

- GABA_A receptors and their clinical variants.
- Disease-causing mechanism of GABA_A variants.
- Therapeutic strategies to correct the function of pathogenic GABA_A receptor variants.

Epilepsy-associated GABA_A Variants

ClinVar

ClinVar

gabra1[gene]

Search

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- Clinical significance
- Conflicting interpretations (24)
- Benign (11)
- Likely benign (8)
- Uncertain significance (139)
- Likely pathogenic (29)
- Pathogenic (18)

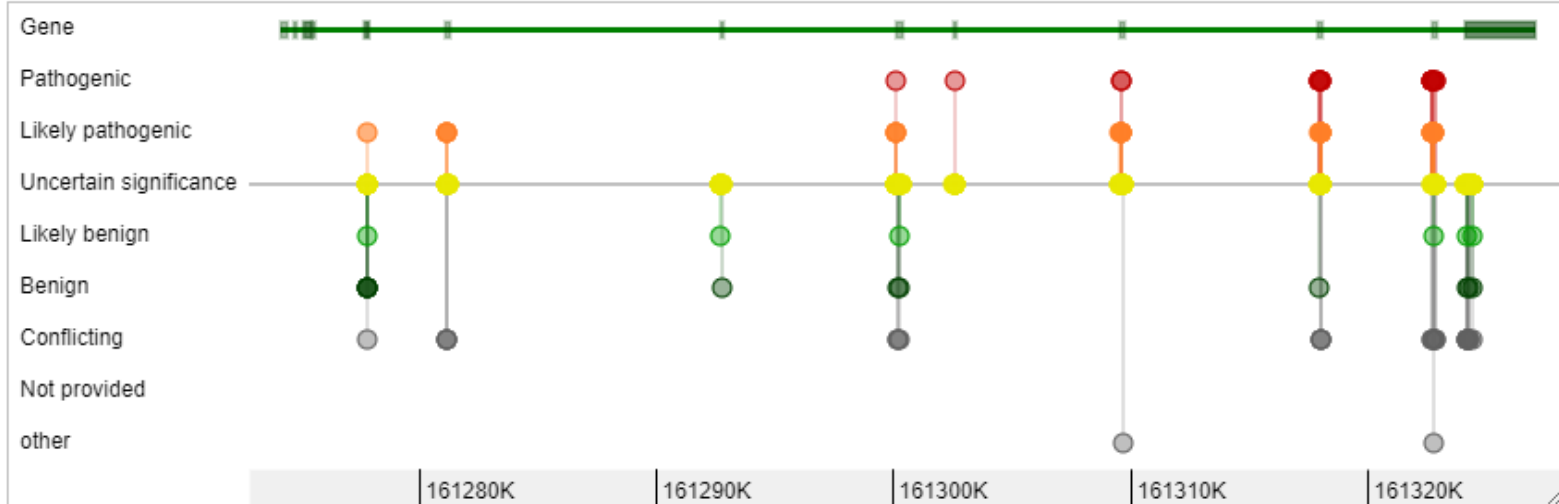
- Types of conflicts
- P/LP vs LB/B (0)
- P/LP vs VUS (13)
- VUS vs LB/B (11)

- Molecular consequence
- Frameshift (0)
- Missense (226)
- Nonsense (0)
- Splice site (0)
- ncRNA (0)
- Near gene (0)
- UTR (0)

Variation type

Graphical view of search results

GRCh37



Search results

Display options Sort by Location Download

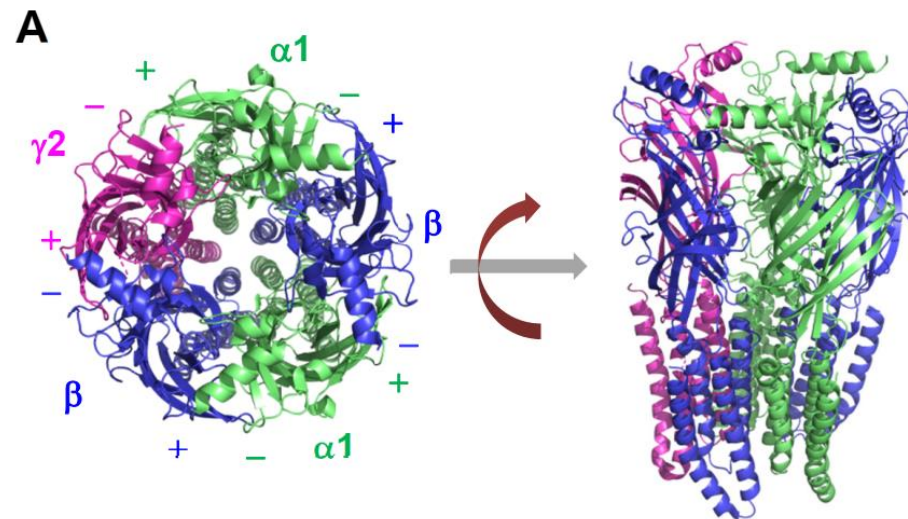
Items: 1 to 100 of 226

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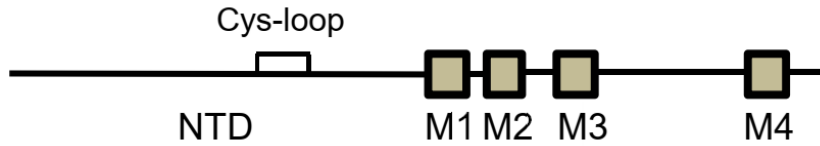
Filters activated: Missense. Clear all to show 590 items.

GABA_A Receptor Missense Variants in NIH ClinVar

GABA _A subunits	Clinical Significance (ClinVar annotation)		
	Pathogenic (including likely)	Uncertain (including conflicting)	Benign
$\alpha 1$	48	168	16
$\beta 2$	39	141	34
$\beta 3$	52	162	18
$\gamma 2$	26	173	12



GABA_A Receptor Variants in NIH ClinVar Database



	α1	loop 1	β1	loop 2	β2	loop D	
alpha1	KDNTTVFTRLDRLLDGYDN	RLRPGLGERV	TEVKTDIFVT	SFGPVSDHDM	EYTIIDVFFRQ		95
beta2	PSNMSLVKET	VDRLKGYDI	RLRPDFGGPP	VAVGMNIDIA	SIDMVSEVNM	DYTLTMYFQQ	89
beta3	PGNMSFVKET	VDKLLKGYDI	RLRPDFGGPP	VCVGMNIDIA	SIDMVSEVNM	DYTLTMYFQQ	90
gamma2	KVPEGDVTVI	LNNLLEGYDN	KLRPDIGVKP	TLIHDTMYVN	SIGPVNAINM	EYTIIDIFFAQ	119

	β2	loop 3	β3	α2	loop 4	loop A (loop 5)	β5	β5'	loop 6	β6	
alpha1	SWKDERLKFEK	GPMTVLRLNN	LMASKIWTED	TEFFHNGKKS	SV	AHNMTMPNKL	LRLITEDG	TLL			155
beta2	AWRDKRLSYN	VIPNLNLTLDN	RVADQLWVPD	TYFLNDKKS	F	VHGVTVKNRM	IRLHPDGT	VL			149
beta3	YWRDKRLAYS	GIPLNLTLDN	RVADQLWVPD	TYFLNDKKS	F	VHGVTVKNRM	IRLHPDGT	VL			150
gamma2	TWYDRRLKFN	STLKVLRLNS	NMVGKIWIPD	TEFFRNSKKAD		AHWITTPNRM	LRIWNDG	VL			179

	loop E	β6	Cys-loop (loop 7)	β7	loop B (loop 8)	β8	loop F	loop 9	
alpha1	YTMRLTVRAE	CPMHLEDFPM	DAHACPLKFG	SYAYTRAEEV	YEWTR	EPARS	VVVAEDGS	RL	215
beta2	YGLRITTTAA	CMDLRRYPL	DEQNCTLEIE	SYGYTTDDIE	FYWRG	DDN	AVTGVTK	IEL	207
beta3	YGLRITTTAA	CMDLRRYPL	DEQNCTLEIE	SYGYTTDDIE	FYWRG	GDK	AVTGVER	IEL	208
gamma2	YTLRLTIDAE	CQLQLHNFPM	DEHSCPLEFS	SYGYPREEIV	YQWKR	SSV	EVG	DRSWRL	237

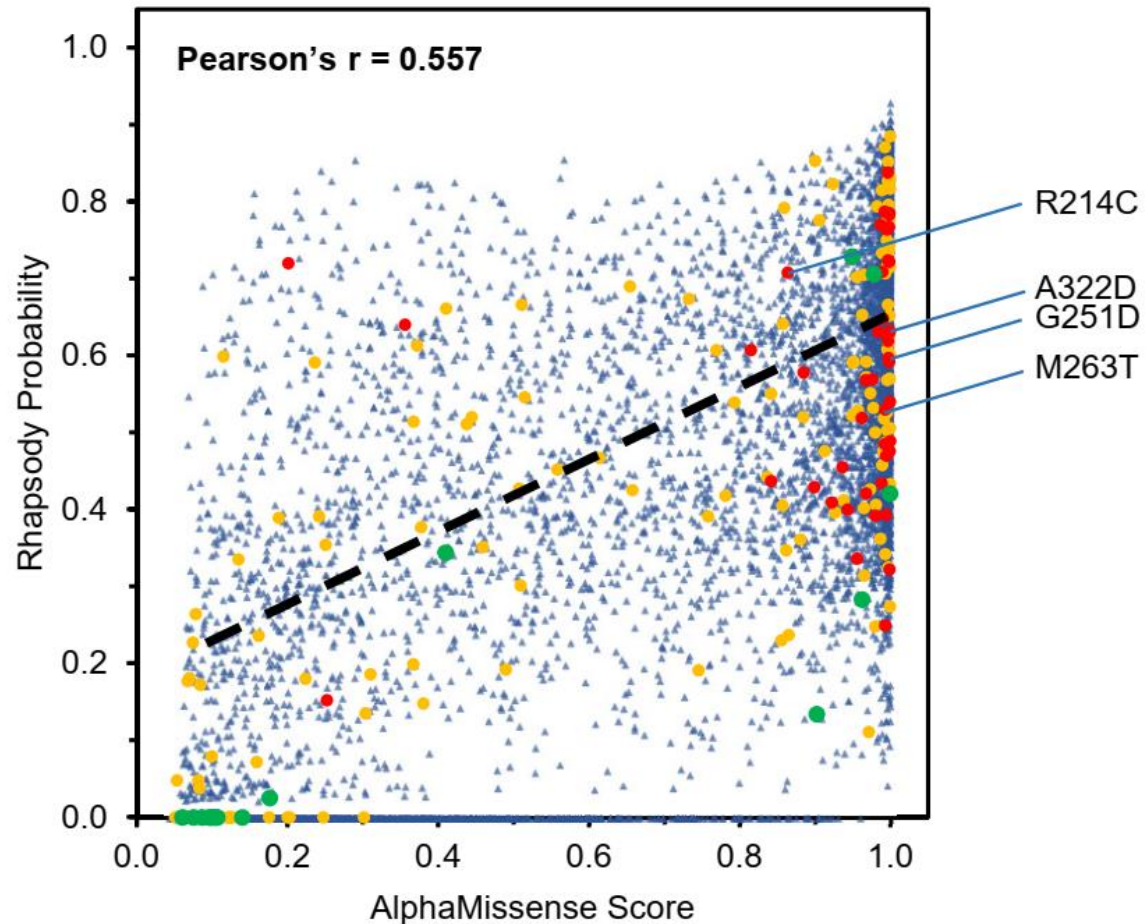
	β9	loop C (loop 10)	β10	M1				
alpha1	NQYDLLGQTV	DSGIVQSSTG	EYVVMTHFH	LKRKIGYFVI	QTYLPCIMTV	ILSQVSFWLN	275	
beta2	PQFSIVDYKL	ITKKVVVFSTG	SYPRLSLSFK	LKRNIGYFIL	QTYMPSILIT	ILSWVSFWIN	267	
beta3	PQFSIVEHRL	VSRNVVFATG	AYPRLSLSF	LKRNIGYFIL	QTYMPSILIT	ILSWVSFWIN	268	
gamma2	YQFSFVGLRN	TTEVVKTTSG	DYVVM	SVYFD	LSRRMGYFTI	QTYIPCTLI	VLSWVSFWIN	297

	M2	M3							
alpha1	RESVPARTVF	GVTTVLMTT	LSISARNSLP	KVAYATAMDW	FIAVCYAFVF	SALIEFATVN	335		
beta2	YDASAARVAL	GITTVLMTT	INTHLRETLP	KIPYVKAIDM	YLMGCFVFVF	MALLEYALVN	327		
beta3	YDASAARVAL	GITTVLMTT	INTHLRETLP	KIPYVKAIDM	YLMGCFVFVF	LALLEYAFVN	328		
gamma2	KDAVPARTSL	GITTVLMTT	LSTIARKSLP	KVS	YVTAMD	L	FVSVCFIFVF	SALVEYGT	357

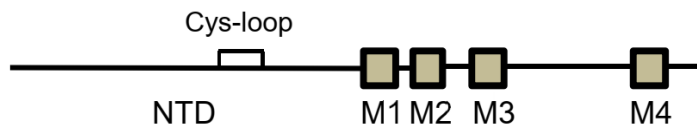
Red: pathogenic;
Yellow: uncertain;
Green: benign.

Pathogenicity Prediction of GABA_A Missense Variants Using Artificial Intelligence-based Algorithm

C



- AlphaMissense
 - based on Alpha-fold structures and evolutionary conservation.
- Rhapsody
 - based on known structures and dynamic information.



Saturating mutagenesis prediction: any missense variant can be predicted.

Pathogenicity Prediction of GABA_A Variants Using Artificial Intelligence-based Algorithm

Tutorial

- Step 1: Go to the following publication:
<https://www.biorxiv.org/content/10.1101/2023.11.14.567135v1.full>
- Step 2: Click “Supplementary Material:

New Results [Follow this preprint](#)

Pathogenicity Prediction of GABA_A Receptor Missense Variants

Ya-Juan Wang, Giang H. Vu, Ting-Wei Mu
doi: <https://doi.org/10.1101/2023.11.14.567135>

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- Step 3: Download the Supplemental Tables to your computer.
 - Table S1 is for $\alpha 1$ subunit.
 - Table S2 is for $\beta 2$ subunit.
 - Table S3 is for $\beta 3$ subunit.
 - Table S4 is for $\gamma 2$ subunit.

New Results

Pathogenicity Prediction of GABA_A Receptor Missense Variants

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Abstract Full Text Info/History Metrics **Supplementary material**

- Supplemental Table S1 | [\[supplements/567135_file03.xlsx\]](#)
- Supplemental Table S2 | [\[supplements/567135_file04.xlsx\]](#)
- Supplemental Table S3 | [\[supplements/567135_file05.xlsx\]](#)
- Supplemental Table S4 | [\[supplements/567135_file06.xlsx\]](#)

Pathogenicity Prediction of GABA_A Variants

Using Artificial Intelligence-based Algorithm

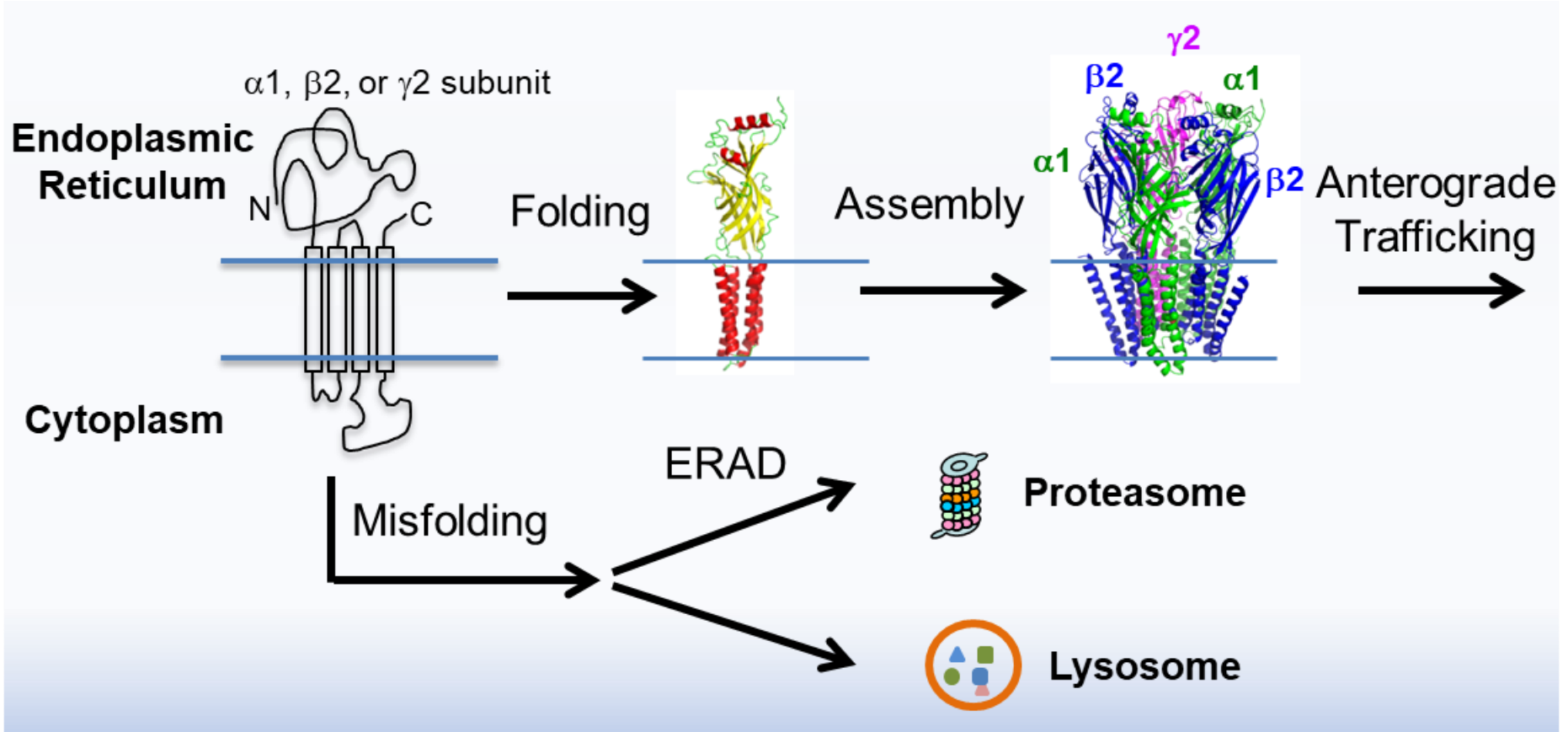
Tutorial

- Step 4: Open the table of your interest: Table S1, α 1 subunit; S2, β 2; S3, β 3; S4, γ 2.

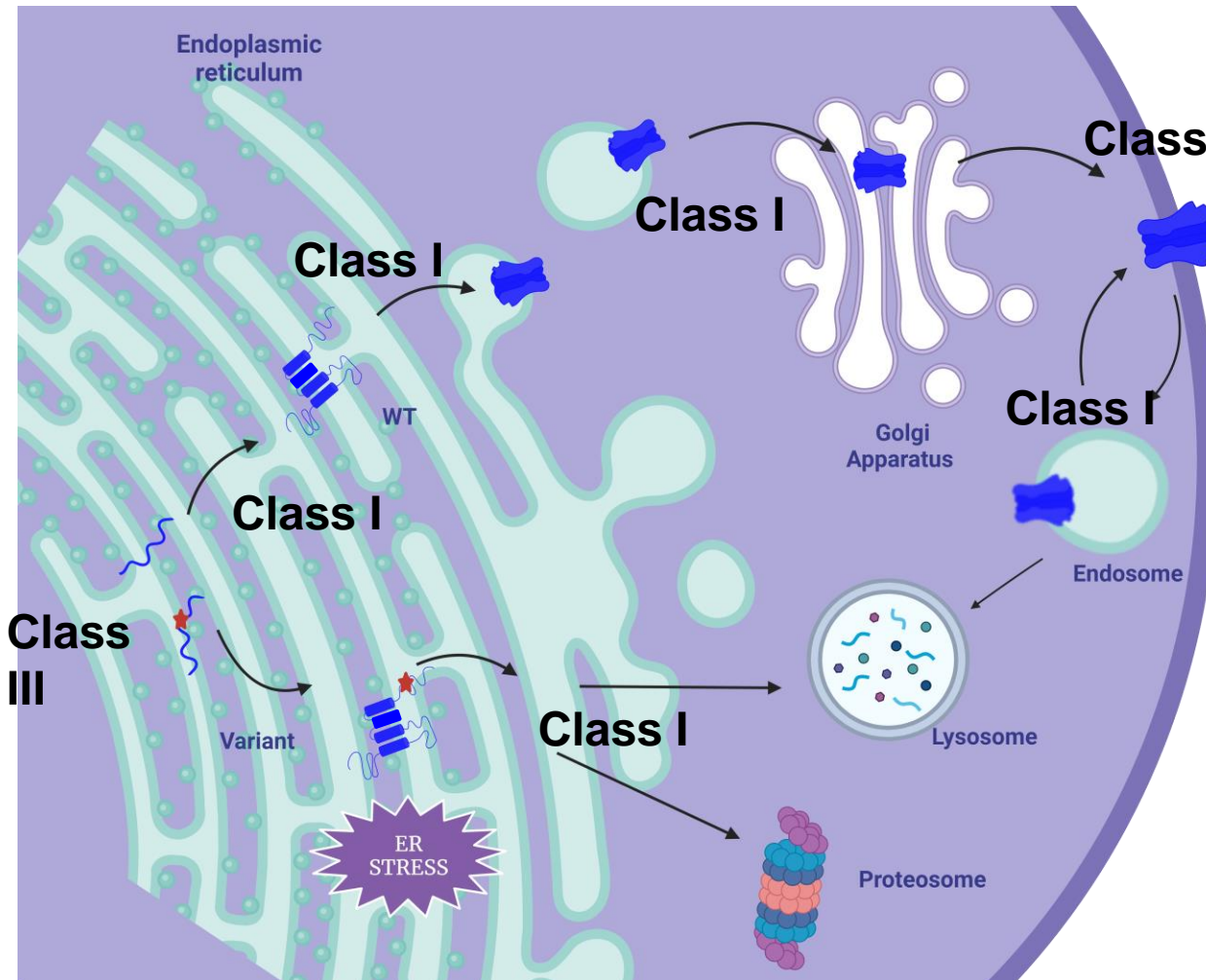
Supplemental Table 1. The α 1 subunit of GABA_AR pathogenicity annotations and predications.

Protein change	AM score	AM prediction	RS prob.	RS class	Name	Condition(s)	Clinical significance (Last reviewed)
R34H	0.9265	pathogenic	0.396	neutral	NM_001127644.2(GABRA1):c.281G>A (p.Arg34H)	Inborn genetic diseases not provided	Uncertain significance(Last reviewed: Sep 1, 2023)
R34S	0.9959	pathogenic	0.503	prob. delet.	NM_001127644.2(GABRA1):c.280C>A (p.Arg34S)	not provided	Uncertain significance(Last reviewed: Dec 10, 2020)
W97R	0.9999	pathogenic	0.885	deleterious	NM_001127644.2(GABRA1):c.289T>A (p.Trp97R)	not provided	Uncertain significance(Last reviewed: Jul 1, 2022)
D99G	0.9988	pathogenic	0.746	deleterious	NM_001127644.2(GABRA1):c.296A>G (p.Asp99D)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Uncertain significance(Last reviewed: Feb 5, 2022)
F104L	0.9995	pathogenic	0.274	neutral	NM_001127644.2(GABRA1):c.312T>G (p.Phe104L)	Epilepsy, childhood absence 4 Epilepsy, idiopathic ge	Uncertain significance(Last reviewed: Aug 28, 2021)
M108V	0.1763	benign	0.025	neutral	NM_001127644.2(GABRA1):c.322A>G (p.Met108V)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Benign(Last reviewed: Feb 3, 2022)
T109I	0.2239	benign	0.18	neutral	NM_001127644.2(GABRA1):c.326C>T (p.Thr109I)	Idiopathic generalized epilepsy Epilepsy, childhood ab	Uncertain significance(Last reviewed: Sep 24, 2021)
L111P	0.9991	pathogenic	0.833	deleterious	NM_001127644.2(GABRA1):c.332T>C (p.Leu111I)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Uncertain significance(Last reviewed: Jul 25, 2022)
R112L	0.8651	pathogenic	0.237	neutral	NM_001127644.2(GABRA1):c.335G>T (p.Arg112L)	Epilepsy, idiopathic generalized, susceptibility to, 13 E	Uncertain significance(Last reviewed: Aug 21, 2022)
R112Q	0.4379	ambiguous	0.511	prob. delet.	NM_001127644.2(GABRA1):c.335G>A (p.Arg112Q)	Inborn genetic diseases Epilepsy, idiopathic generaliz	Conflicting interpretations of pathogenicity(Last reviewed: Dec 1, 2022)
R112W	0.857	pathogenic	0.405	neutral	NM_001127644.2(GABRA1):c.334C>T (p.Arg112W)	Idiopathic generalized epilepsy Epilepsy, childhood ab	Conflicting interpretations of pathogenicity(Last reviewed: May 10, 2023)
L113F	0.993	pathogenic	0.342	neutral	NM_001127644.2(GABRA1):c.339A>T (p.Leu113F)	Inborn genetic diseases	Uncertain significance(Last reviewed: Mar 14, 2017)
P124L	0.9982	pathogenic	0.767	deleterious	NM_001127644.2(GABRA1):c.371C>T (p.Pro124L)	Epilepsy, idiopathic generalized, susceptibility to, 13	Likely pathogenic(Last reviewed: Mar 22, 2023)
D125G	0.9991	pathogenic	0.57	deleterious	NM_001127644.2(GABRA1):c.374A>G (p.Asp125D)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Uncertain significance(Last reviewed: Aug 27, 2021)
F127V	0.968	pathogenic	0.592	deleterious	NM_001127644.2(GABRA1):c.379T>G (p.Phe127V)	Epilepsy, idiopathic generalized, susceptibility to, 13 E	Uncertain significance(Last reviewed: Jun 8, 2022)
H129Y	0.2507	benign	0.354	neutral	NM_001127644.2(GABRA1):c.385C>T (p.His129Y)	Idiopathic generalized epilepsy Epilepsy, childhood ab	Uncertain significance(Last reviewed: Aug 9, 2022)
A136S	0.3673	ambiguous	0.514	prob. delet.	NM_001127644.2(GABRA1):c.406G>T (p.Ala136S)	Developmental and epileptic encephalopathy, 19	Uncertain significance(Last reviewed: Feb 14, 2020)
M141R	0.9022	pathogenic	0.134	neutral	NM_001127644.2(GABRA1):c.422T>G (p.Met141R)	Epilepsy, childhood absence 4 Idiopathic generalized	Benign(Last reviewed: Mar 5, 2021)
K144E	0.993	pathogenic	0.487	prob. delet.	NM_001127644.2(GABRA1):c.430A>G (p.Lys144E)	Epilepsy, idiopathic generalized, susceptibility to, 13 Id	Uncertain significance(Last reviewed: Feb 10, 2022)
R147G	0.993	pathogenic	0.707	deleterious	NM_001127644.2(GABRA1):c.439C>G (p.Arg147G)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Uncertain significance(Last reviewed: May 6, 2022)
R147Q	0.9003	pathogenic	0.853	deleterious	NM_001127644.2(GABRA1):c.440G>A (p.Arg147Q)	Developmental and epileptic encephalopathy, 19 Idi	Conflicting interpretations of pathogenicity(Last reviewed: Jun 19, 2023)
R147W	0.9058	pathogenic	0.776	deleterious	NM_001127644.2(GABRA1):c.439C>T (p.Arg147W)	Epilepsy, childhood absence 4 Idiopathic generalized	Conflicting interpretations of pathogenicity(Last reviewed: Jan 5, 2023)
I148F	0.9493	pathogenic	0.522	prob. delet.	NM_001127644.2(GABRA1):c.442A>T (p.Ile148F)	not specified	Uncertain significance(Last reviewed: Mar 4, 2015)
G152S	0.9889	pathogenic	0.815	deleterious	NM_001127644.2(GABRA1):c.454G>A (p.Gly152S)	not provided	Uncertain significance(Last reviewed: Jan 17, 2022)
M158I	0.989	pathogenic	0.458	prob. neutra	NM_001127644.2(GABRA1):c.474G>A (p.Met158I)	Epilepsy, childhood absence 4 Idiopathic generalized	Uncertain significance(Last reviewed: Oct 3, 2022)
M158V	0.7812	pathogenic	0.418	neutral	NM_001127644.2(GABRA1):c.472A>G (p.Met158V)	not specified	Uncertain significance(Last reviewed: Aug 15, 2023)
R159T	0.9995	pathogenic	0.506	prob. delet.	NM_001127644.2(GABRA1):c.476G>C (p.Arg159T)	not provided	Uncertain significance(Last reviewed: Jan 24, 2020)
L161K	0.9973	pathogenic	0.836	deleterious	NM_001127644.2(GABRA1):c.482C>A (p.Thr161K)	not provided	Uncertain significance(Last reviewed: May 16, 2022)
V162G	0.9713	pathogenic	0.111	neutral	NM_001127644.2(GABRA1):c.485T>G (p.Val162G)	Epilepsy, idiopathic generalized, susceptibility to, 13 Id	Uncertain significance(Last reviewed: Dec 19, 2021)
V162M	0.7582	pathogenic	0.391	neutral	NM_001127644.2(GABRA1):c.484G>A (p.Val162M)	not provided	Uncertain significance(Last reviewed: Jan 14, 2015)
R163K	0.1618	benign	0.236	neutral	NM_001127644.2(GABRA1):c.488G>A (p.Arg163K)	not provided	Uncertain significance(Last reviewed: Sep 6, 2019)
C166W	0.9996	pathogenic	0.715	deleterious	NM_001127644.2(GABRA1):c.498T>G (p.Cys166W)	Epilepsy, childhood absence 4 Idiopathic generalized	Uncertain significance(Last reviewed: Aug 30, 2021)
P167S	0.6544	pathogenic	0.693	deleterious	NM_001127644.2(GABRA1):c.499C>T (p.Pro167S)	not provided	Uncertain significance(Last reviewed: Jun 1, 2018)
H178Q	0.9515	pathogenic	0.592	deleterious	NM_001127644.2(GABRA1):c.534T>G (p.His178Q)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Uncertain significance(Last reviewed: Sep 1, 2021)
G185V	0.9957	pathogenic	0.568	deleterious	NM_001127644.2(GABRA1):c.554G>T (p.Gly185V)	Epilepsy, childhood absence 4 Idiopathic generalized	Uncertain significance(Last reviewed: Aug 14, 2021)
Y187C	0.965	pathogenic	0.705	deleterious	NM_001127644.2(GABRA1):c.560A>G (p.Tyr187C)	Epilepsy, idiopathic generalized, susceptibility to, 13 Id	Uncertain significance(Last reviewed: Apr 23, 2022)
Y187D	0.9836	pathogenic	0.631	deleterious	NM_001127644.2(GABRA1):c.559T>G (p.Tyr187D)	Epilepsy, idiopathic generalized, susceptibility to, 13	Pathogenic(Last reviewed: May 4, 2022)
Y187F	0.4446	ambiguous	0.52	prob. delet.	NM_001127644.2(GABRA1):c.560A>T (p.Tyr187F)	Epilepsy, idiopathic generalized, susceptibility to, 13 E	Uncertain significance(Last reviewed: Aug 31, 2021)
A188D	0.9985	pathogenic	0.592	deleterious	NM_001127644.2(GABRA1):c.563C>A (p.Ala188D)	Idiopathic generalized epilepsy Epilepsy, idiopathic ge	Likely pathogenic(Last reviewed: Apr 24, 2022)

Protein Quality Control of GABA_A Receptors in the Endoplasmic Reticulum



Classification of GABA_A Variants According to Molecular Functions



Class I: Proteostasis defect.

Folding, assembly, degradation, aggregation, trafficking, endocytosis.

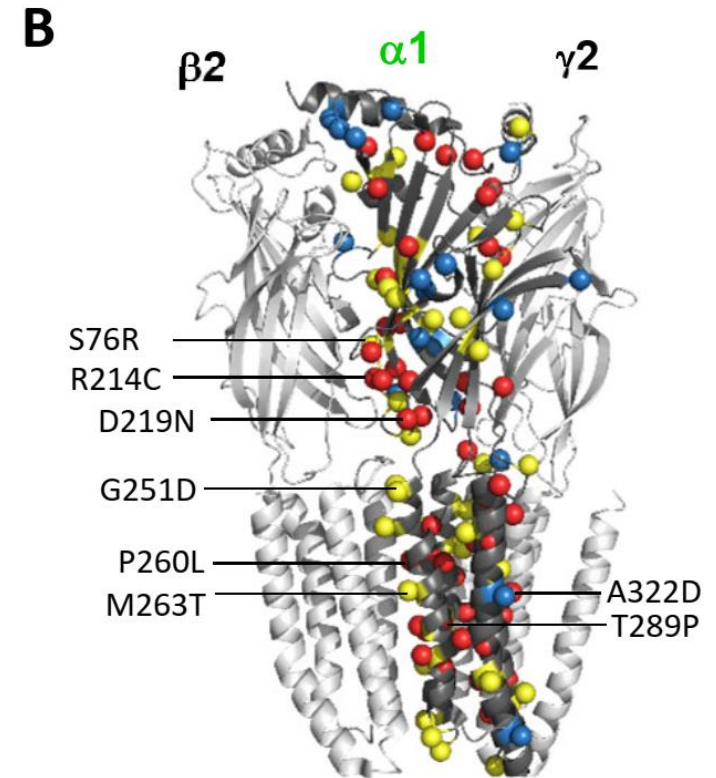
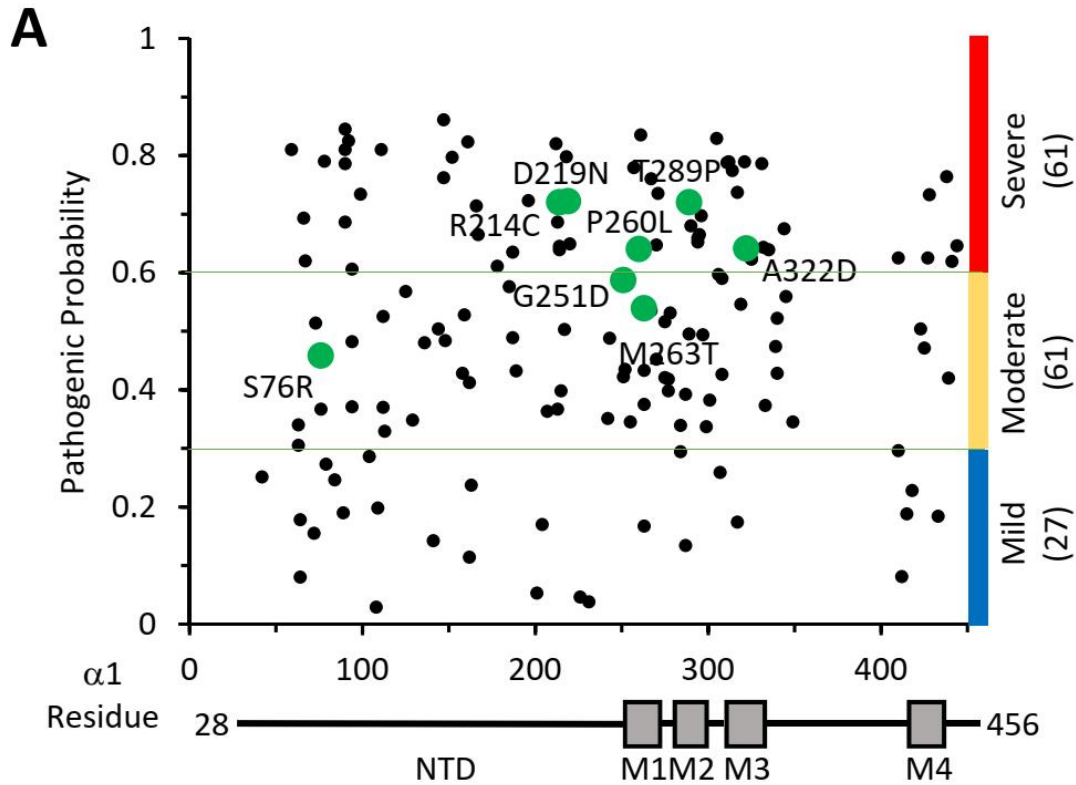
Class II: Electrophysiology defect.

Ligand binding, channel gating, current kinetics.

Class III: Nonsense and frameshift.

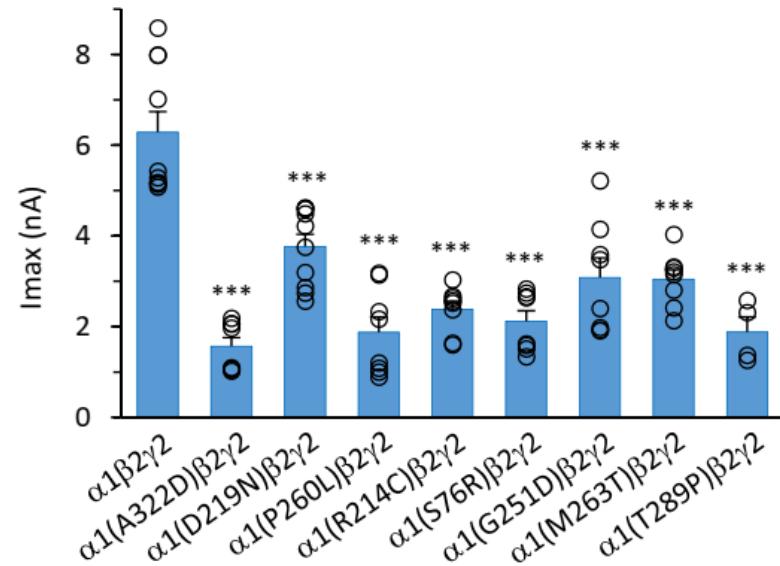
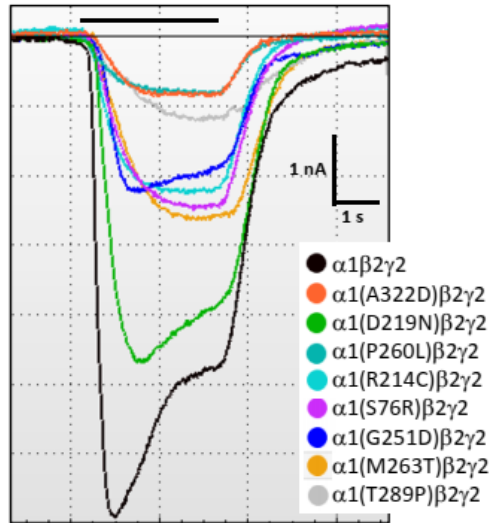
Class IV: Others

Epilepsy-associated GABA_A receptor α 1 variants

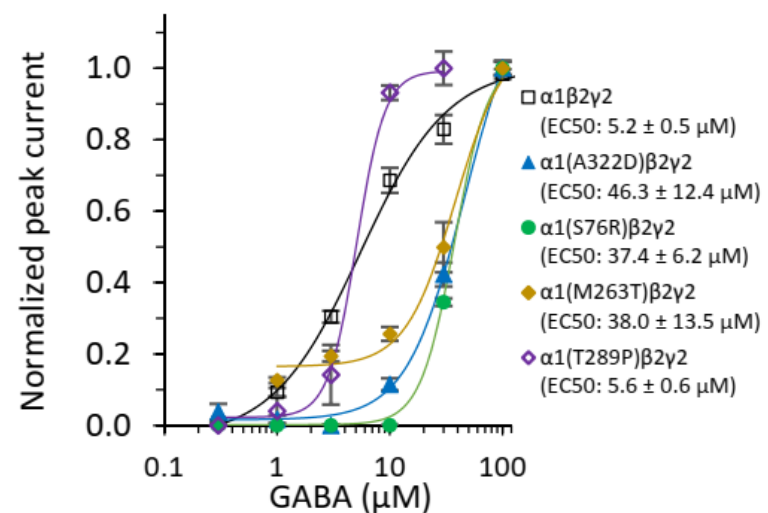
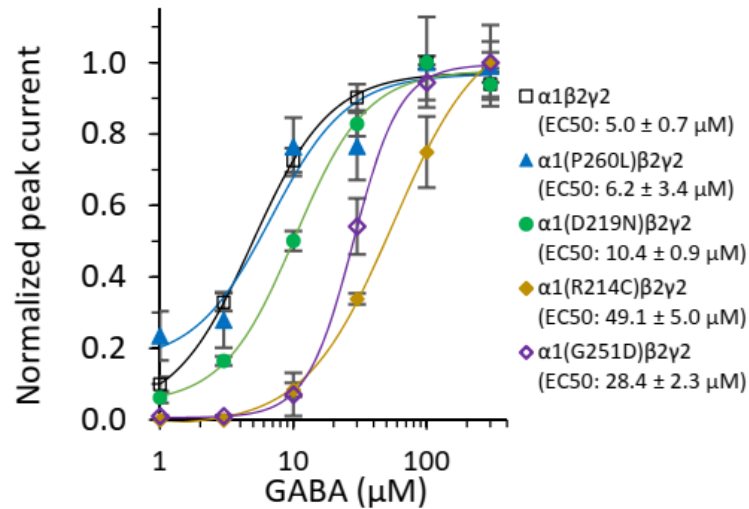


All selected $\alpha 1$ variations cause loss of function of GABA_A receptors

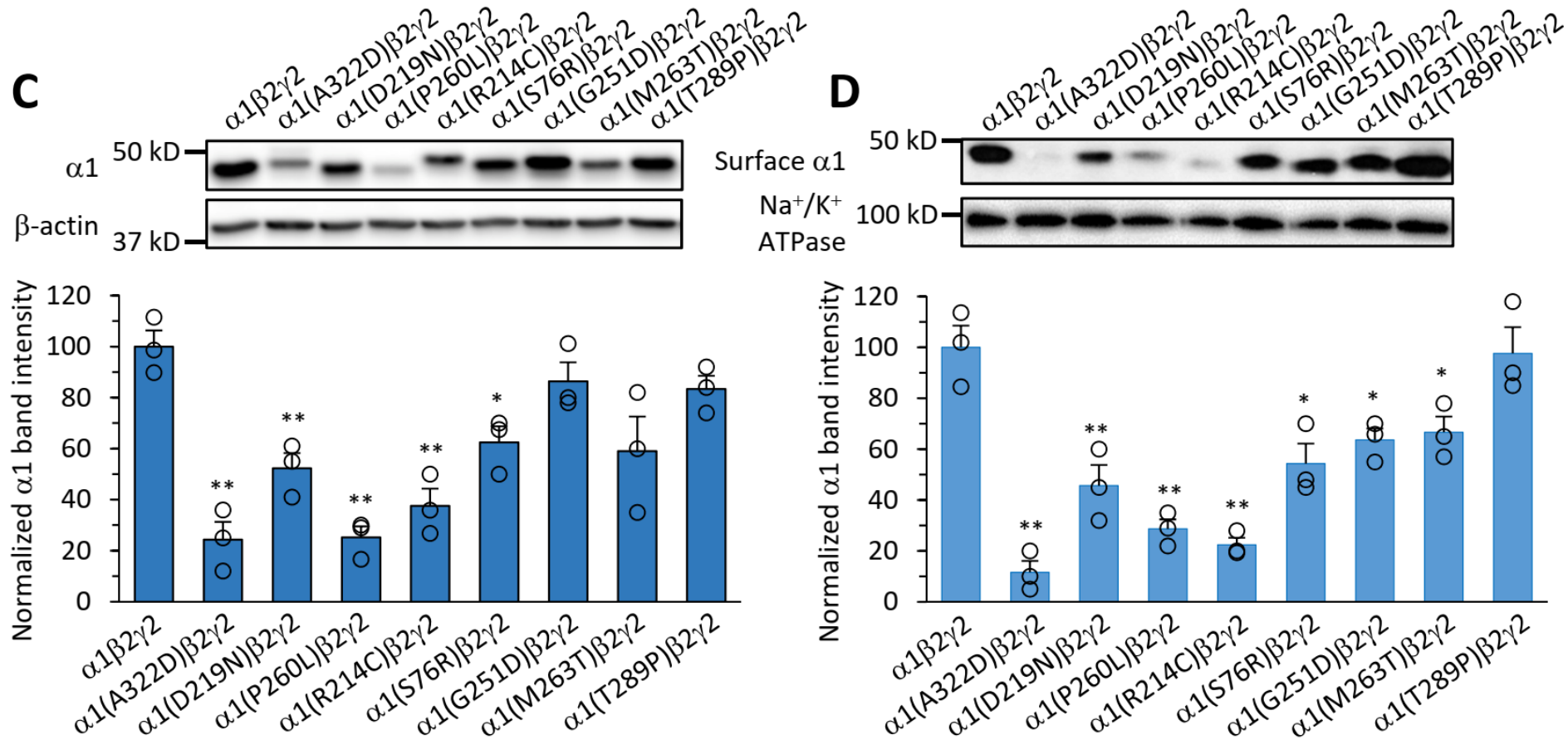
A



B

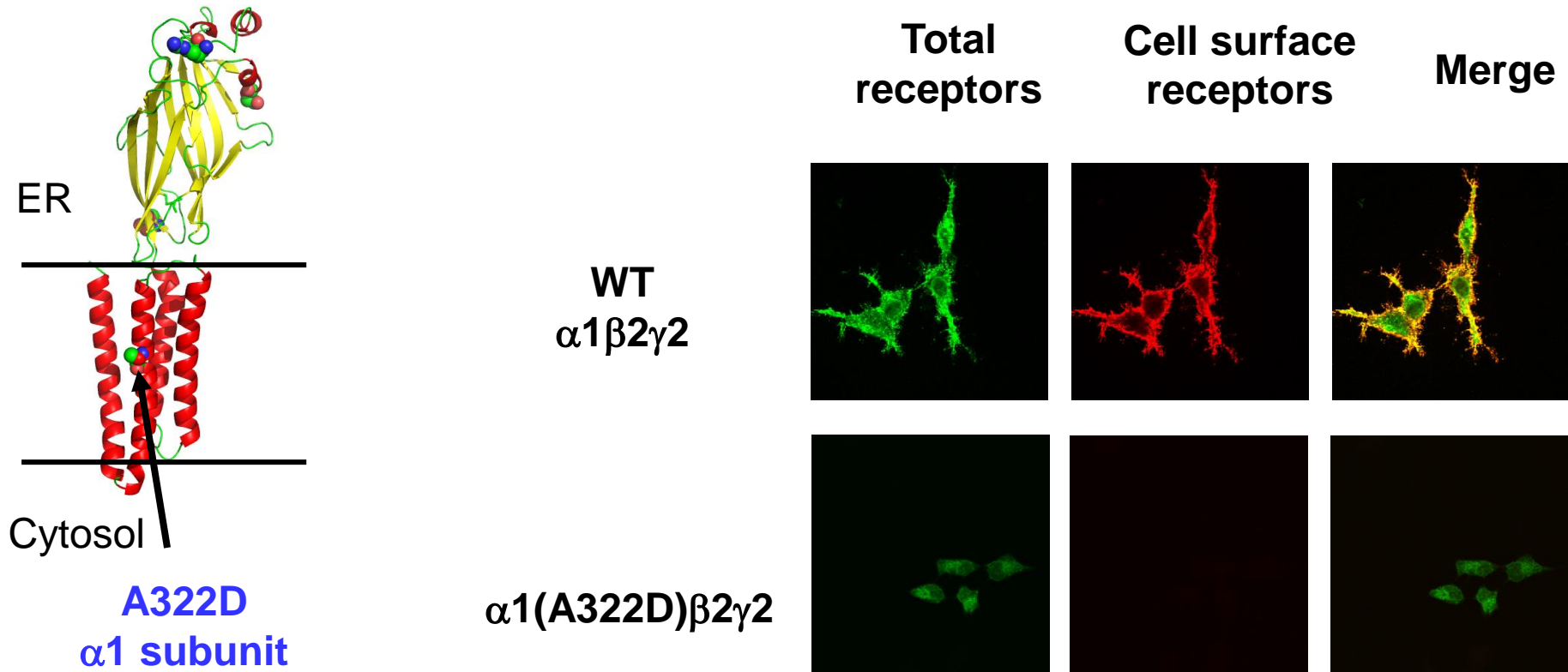


Most selected $\alpha 1$ variations reduce their trafficking to the cell surface



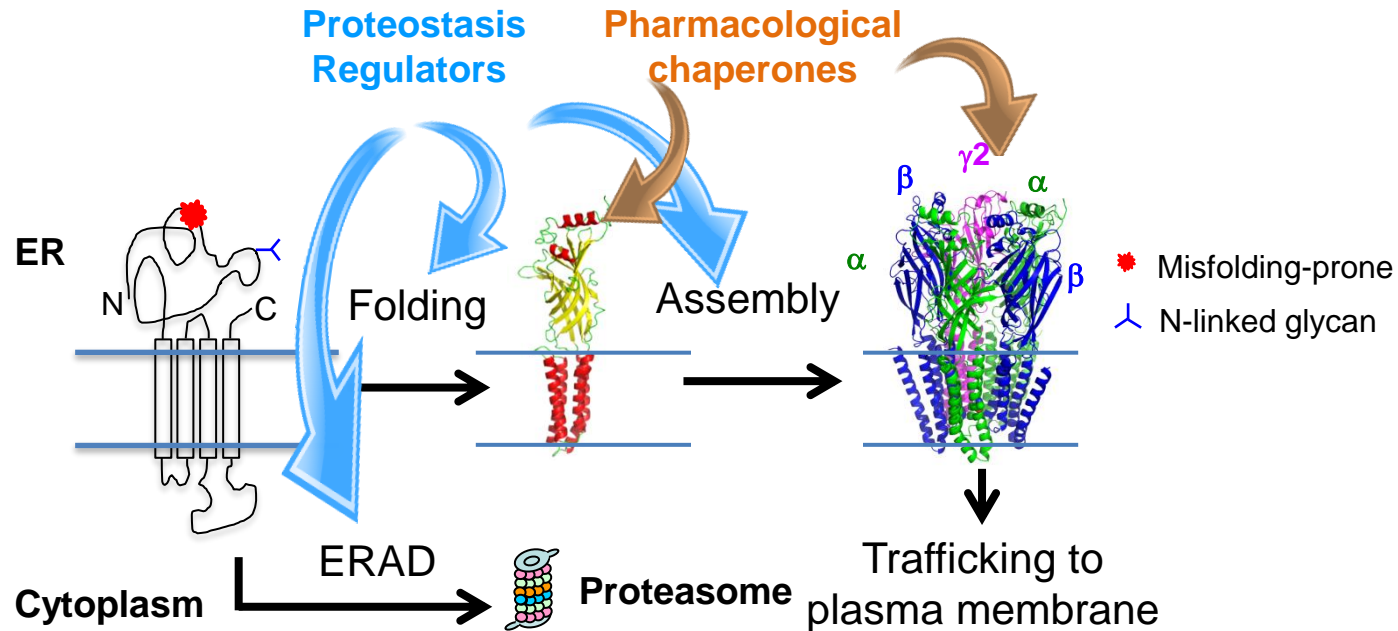
Wang YJ, Seibert H, Ahn LY, Schaffer AE, Mu TW. Pharmacological chaperones restore proteostasis of epilepsy-associated GABA_A receptor variants. bioRxiv 2023. doi: 10.1101/2023.04.18.537383. PMID: 37131660.

Epilepsy-Associated GABA_A variants are Subject to ER-Associated Degradation



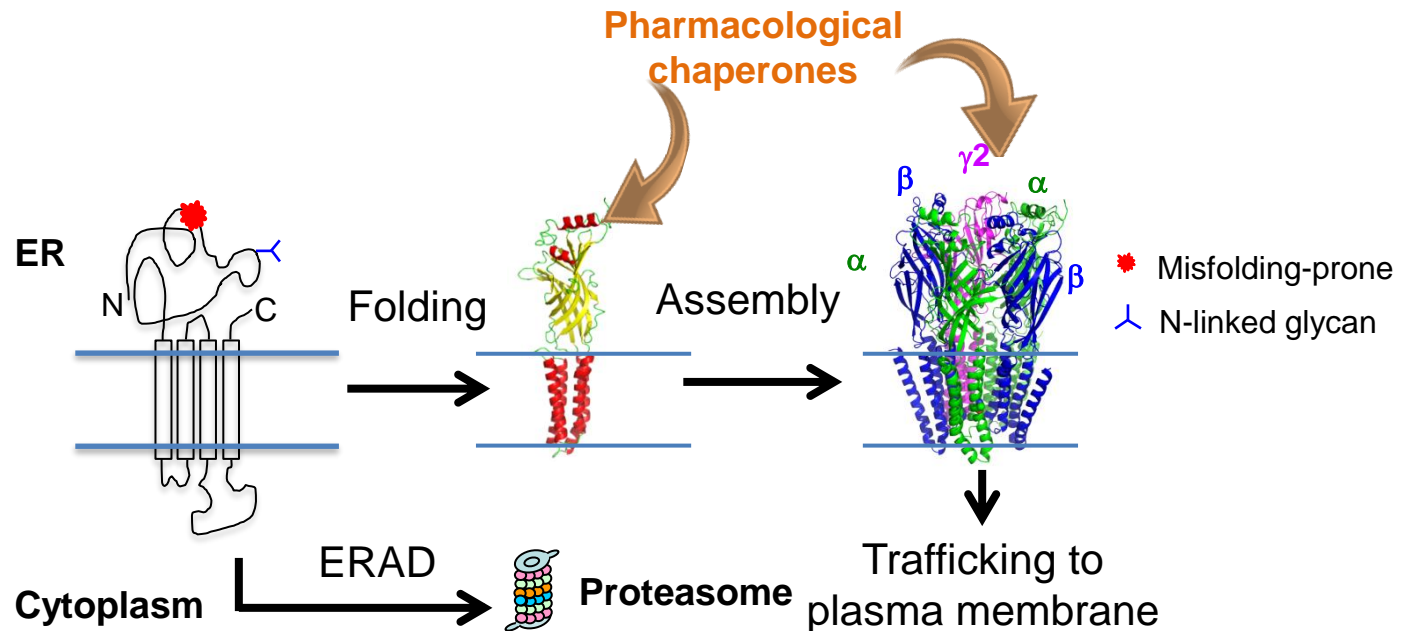
A missense mutation (A322D) in TM3 region of α1 subunit causes misfolding and degradation of the α1 subunits, leading to an autosomal dominant form of juvenile myoclonic epilepsy (ADJME).

A Pharmacological Chaperoning Strategy to Correct GABA_A Variant Function



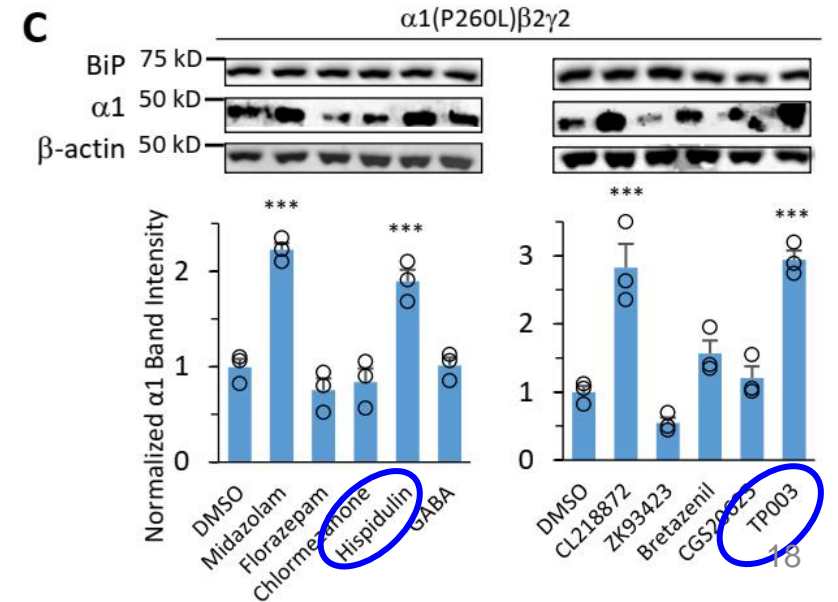
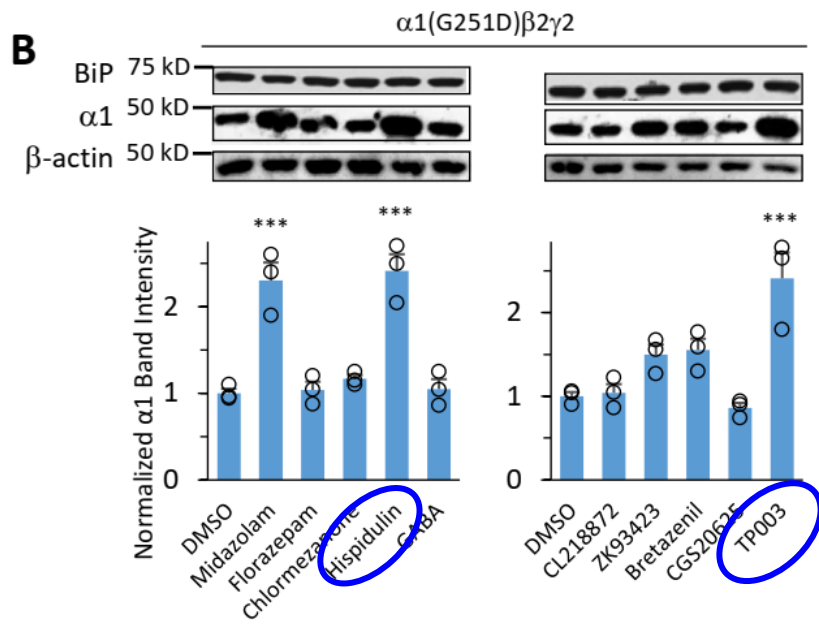
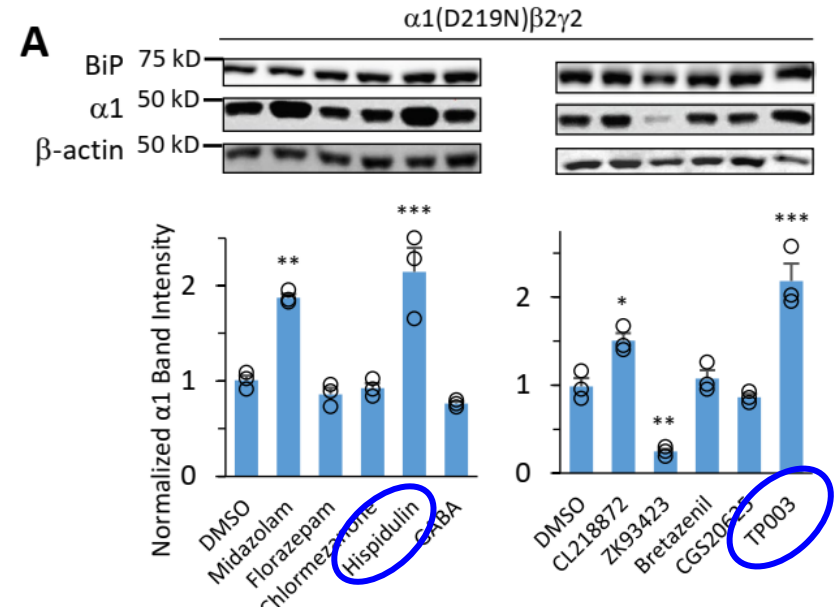
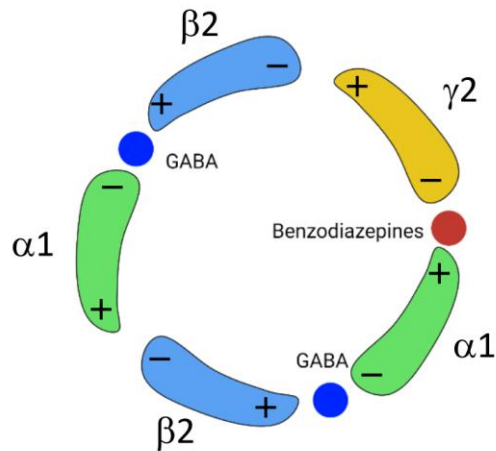
- Proteostasis regulators: folding enhancers.
- Pharmacological chaperones: receptors-specific.
 - Direct GABA_A receptor binders.

Pharmacological Chaperone Strategy to Restore GABA_A function

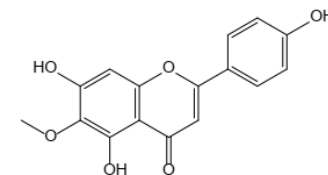
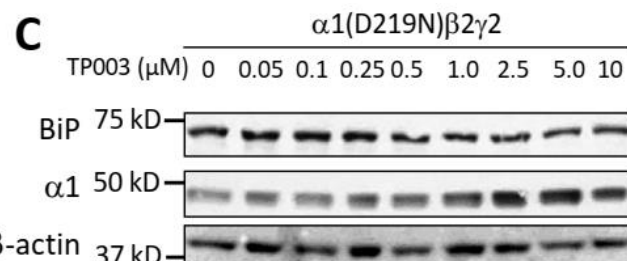
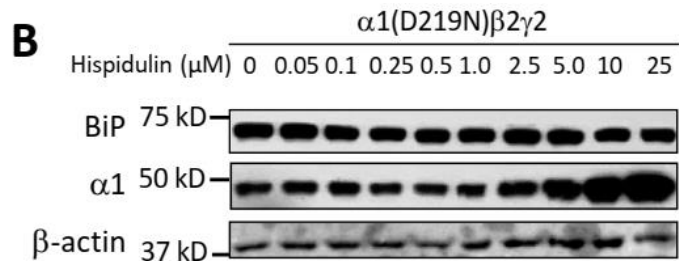


Pharmacological chaperones directly bind GABA_A receptors to stabilize them, and thus promote their forward trafficking.

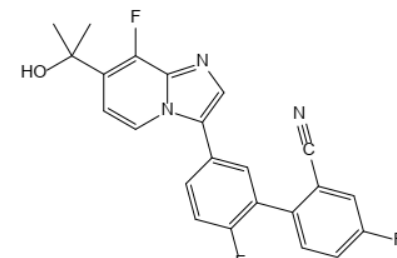
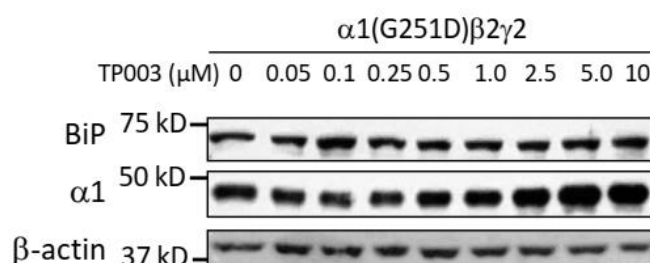
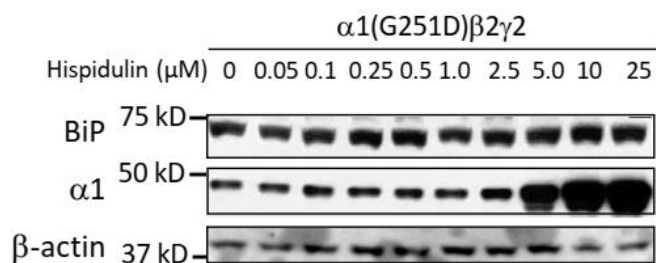
Screening of GABA_A Regulators Identified Effective Pharmacological Chaperones



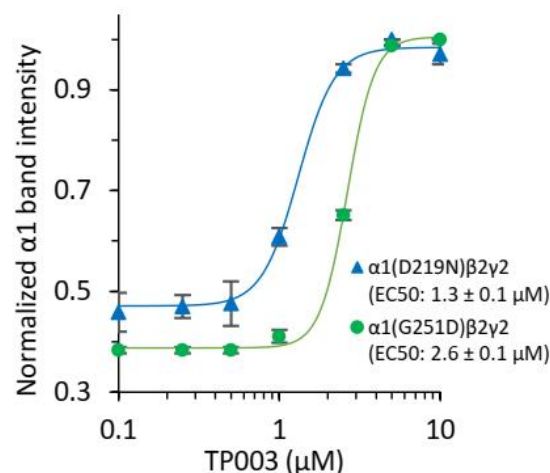
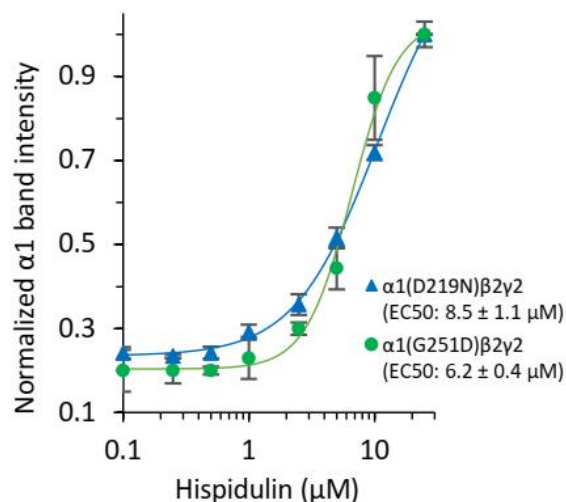
Hispidulin and TP003 Increase the protein levels of pathogenic GABA_A variants



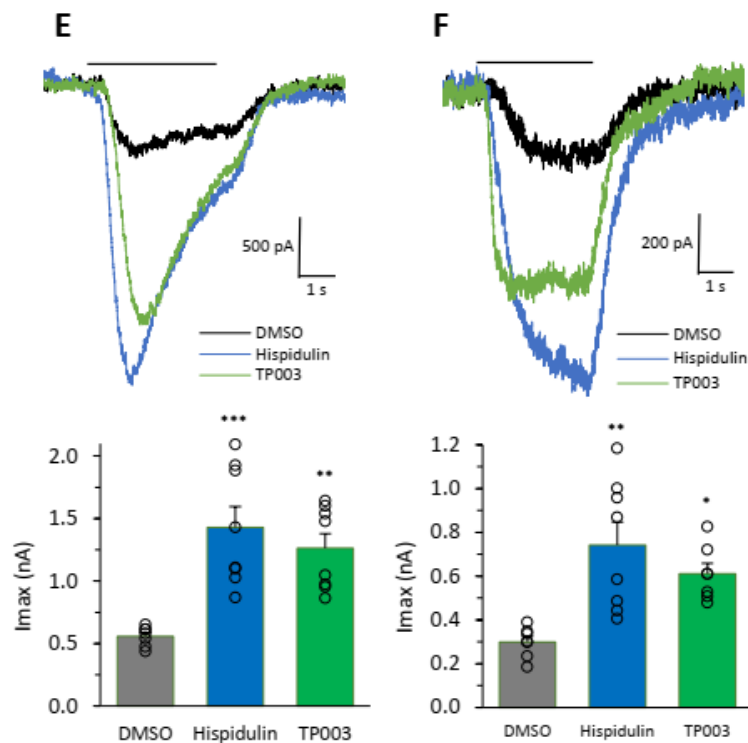
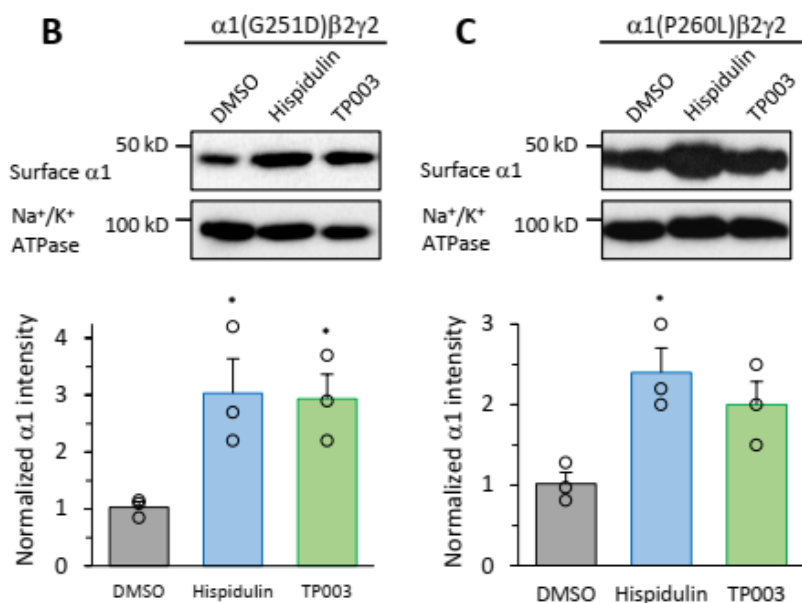
Hispidulin



TP003



Hispidulin and TP003 Enhance the Functional Surface levels of pathogenic GABA_A variants



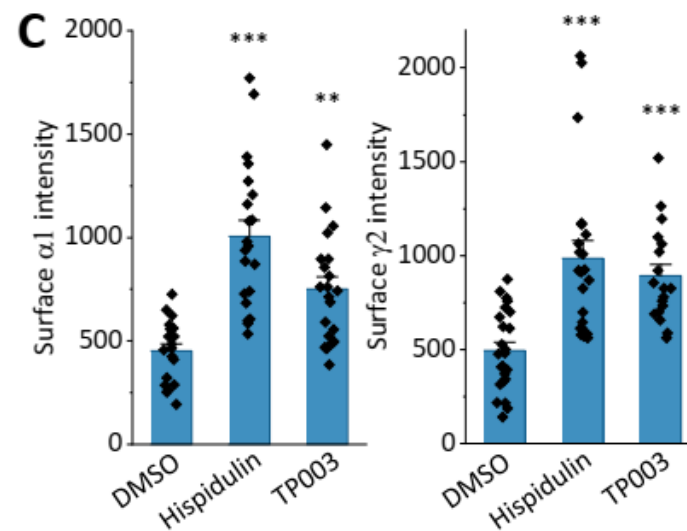
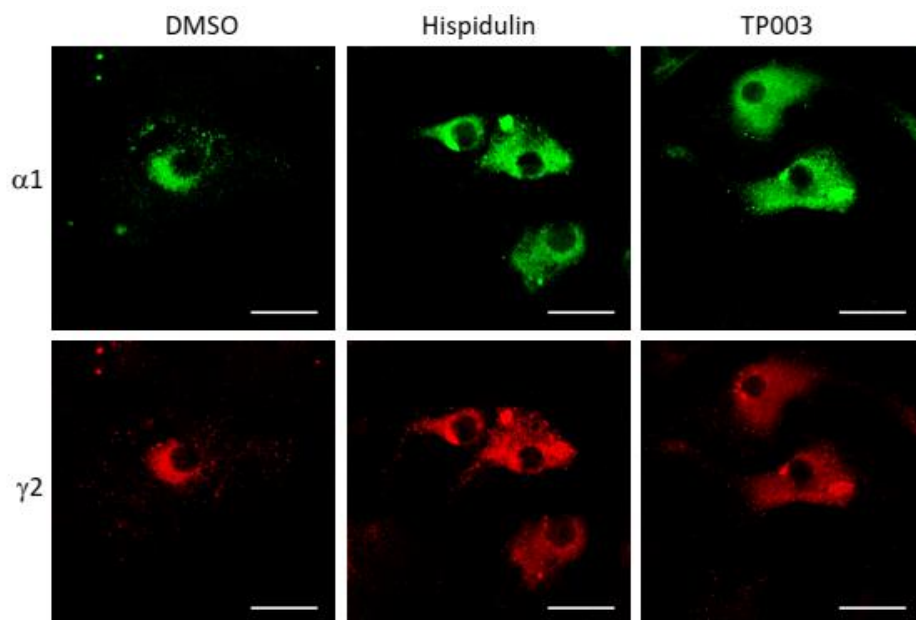
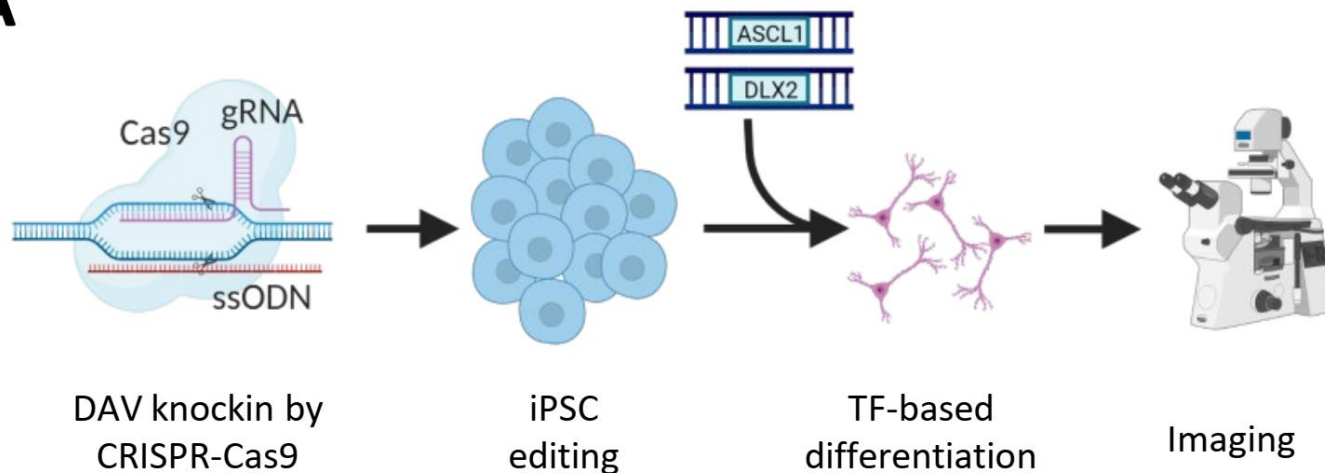
Surface Biotinylation Assay

Patch-Clamping Electrophysiology

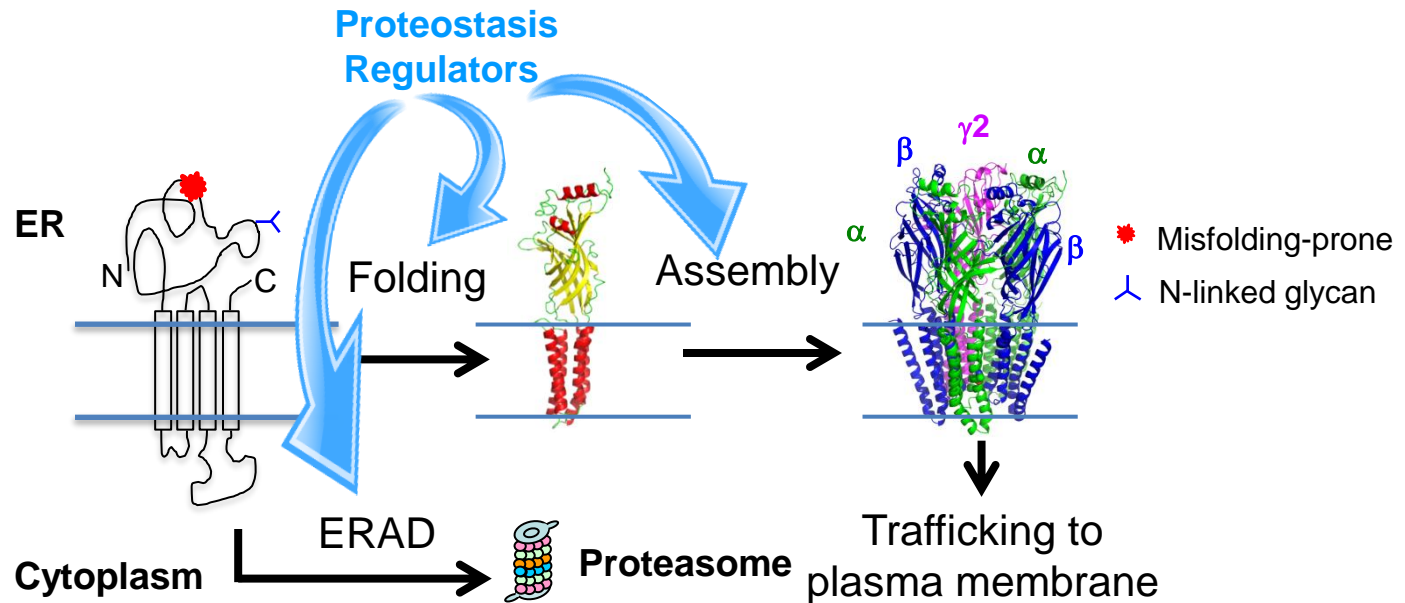
Wang YJ, Seibert H, Ahn LY, Schaffer AE, Mu TW. Pharmacological chaperones restore proteostasis of epilepsy-associated GABA_A receptor variants. *bioRxiv*, 2023, doi: <https://doi.org/10.1101/2023.04.18.537383>. PMID: 37131660

Hispidulin and TP003 Enhance the Surface Staining of pathogenic GABA_A variants in iPSC-derived Neurons

A



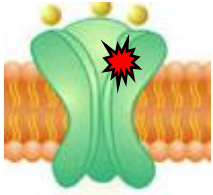
Proteostasis Regulator Strategy to Restore GABA_A function



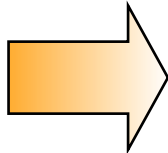
- Activators of the unfolded protein response (UPR)
- Ca²⁺ channel regulators, such as verapamil.
- **FDA-approved drugs, such as dinoprost and dihydroergocristine.**

High-throughput Screening to Identify Proteostasis Regulators

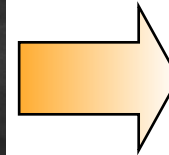
GABA_AR



Expressed in
• HEK cells...

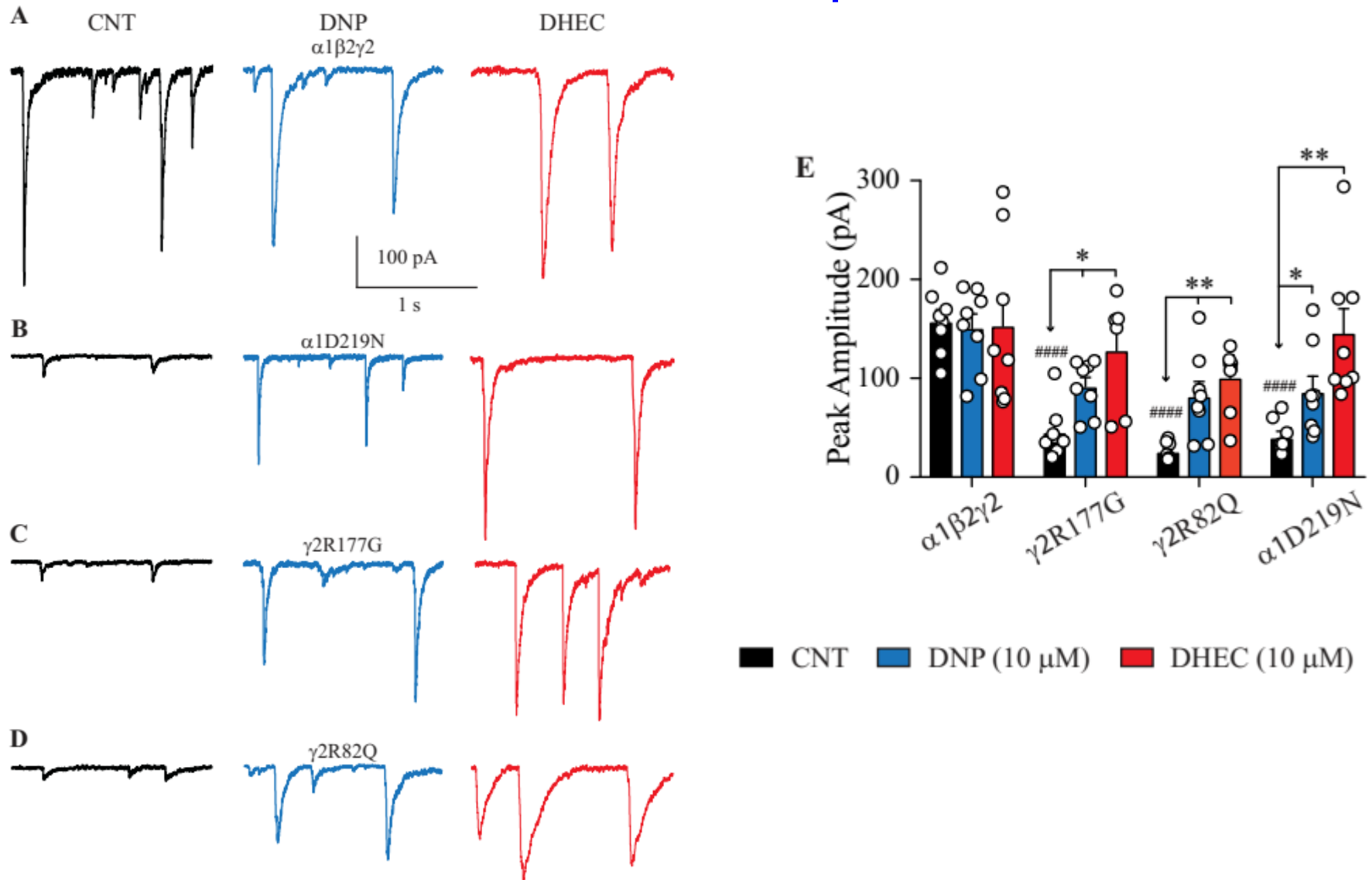


**FDA-approved drug
library screening**



Chemiluminescence
Assay to quantify
GABA_AR total
expression level

DNP and DHEC Increase the Peak Current of Mutant Receptors



Summary

- Reduced surface trafficking and loss of function of GABA_A receptors is a major disease-causing mechanism for GABA_A variants.
- Restoring proteostasis pharmacologically corrects the surface expression and function of pathogenic GABA_A receptors, representing a promising therapeutic strategy to treat GABA_A variant-related genetic epilepsy.
- Several lead compounds, such as hispidulin and dihydroergocristine, were identified as effective reagents to correct the function of GABA_A variants. Their further development is needed for translational application.

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- Adrian Palumbo (undergraduate)
- Shahyan Khan (undergraduate)
- Anjali Jawa (high school student)

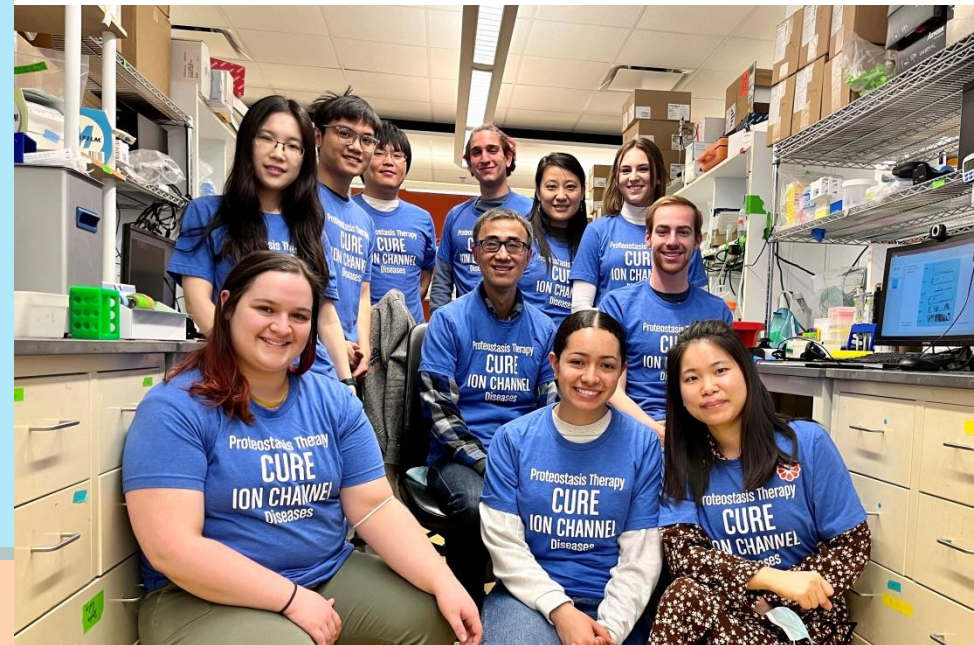
Lab Alumni

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- Dr. Dongyun Han
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- Yingying Yang (Technician)
- Hailey Seibert (Undergraduate)
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