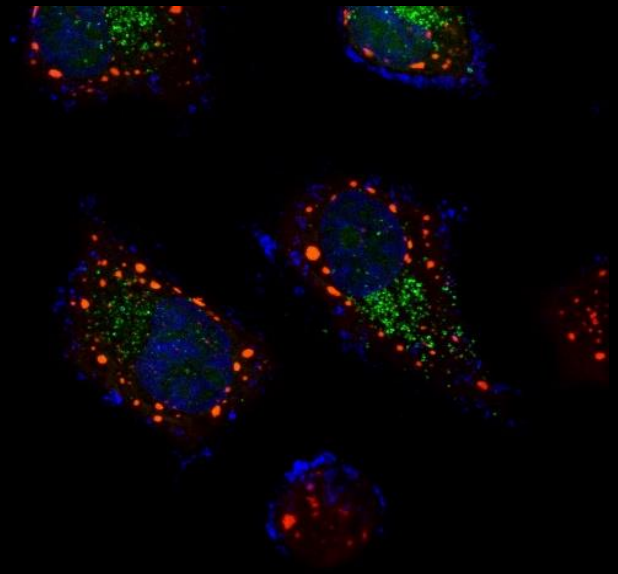
Neuronal Homeostasis in Health & Diseases



Jin-A Lee, Ph.D

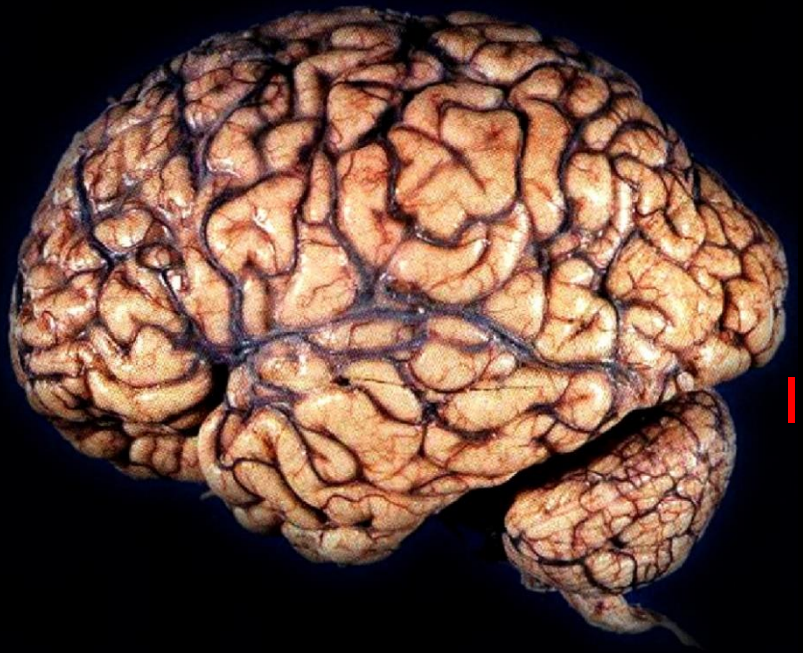
Hannam University

Outline

- *Overview of current research and interests*
- *Topics of fields to explore further*
- *Ideas for potential Korea-EU collaboration under Horizon Europe*

What's Wrong with Your Brain?

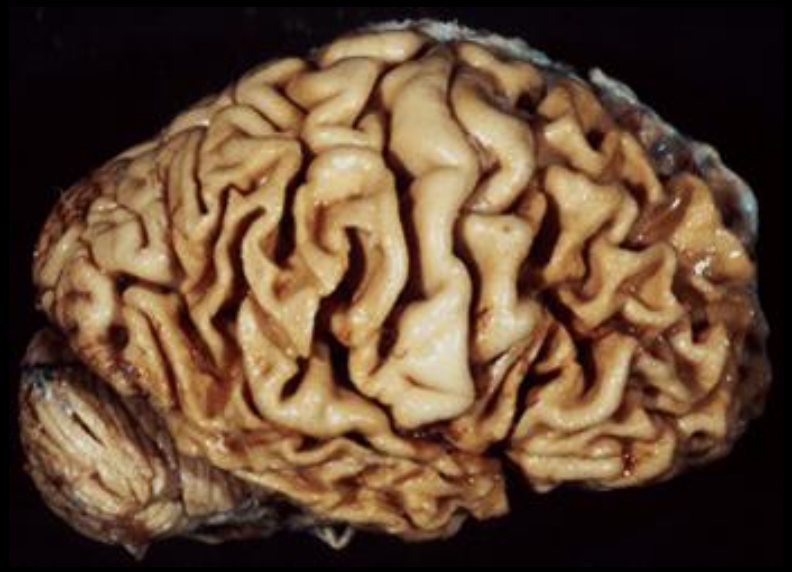
Healthy Brain



Stress

Imbalance

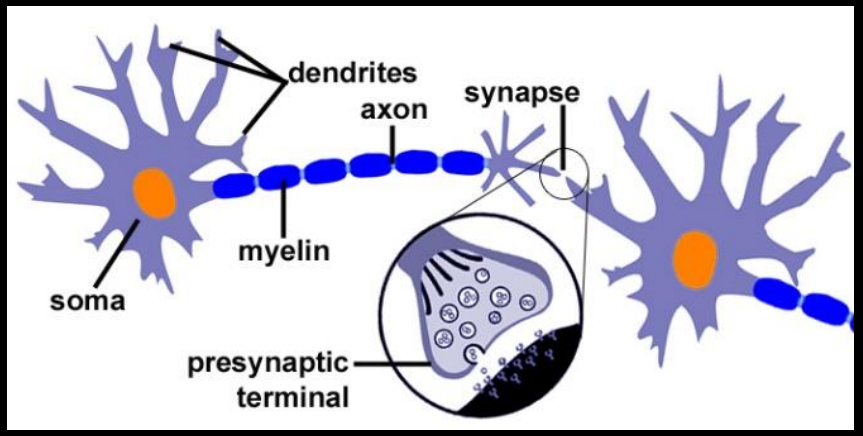
Disease Brain



Cellular quality control systems maintain brain health

Quality Control in the Post-mitotic Neuron

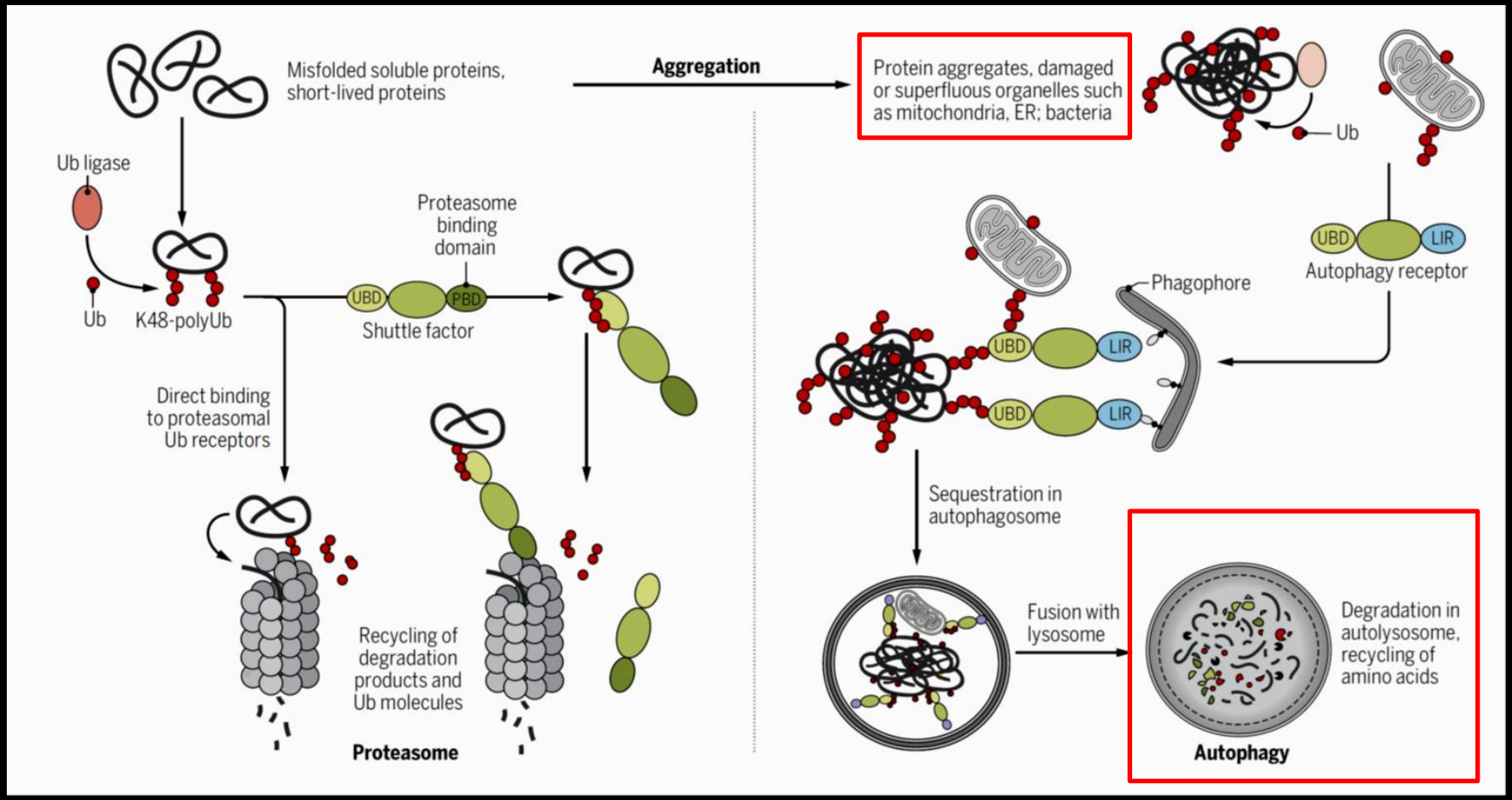
15 Nov. 2019. Special issue, Science



- Post-mitotic nature
- Highly polarized structure
- Dynamic signaling in synapse
- Enriched mitochondria in axon
- Transport of many receptors

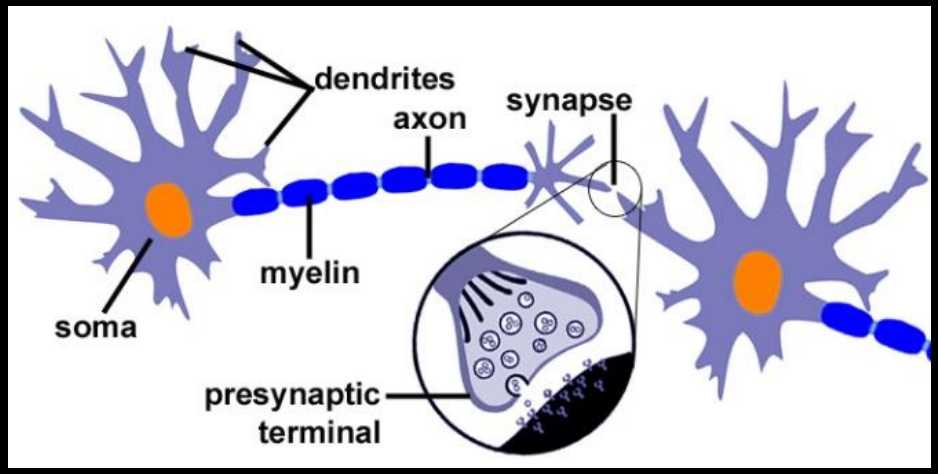
UPS vs Autophagy

Quality control system in the post-mitotic neurons



Pohl & Dikic, 2019, *Science*

Autophagy Safeguards Neuronal Homeostasis

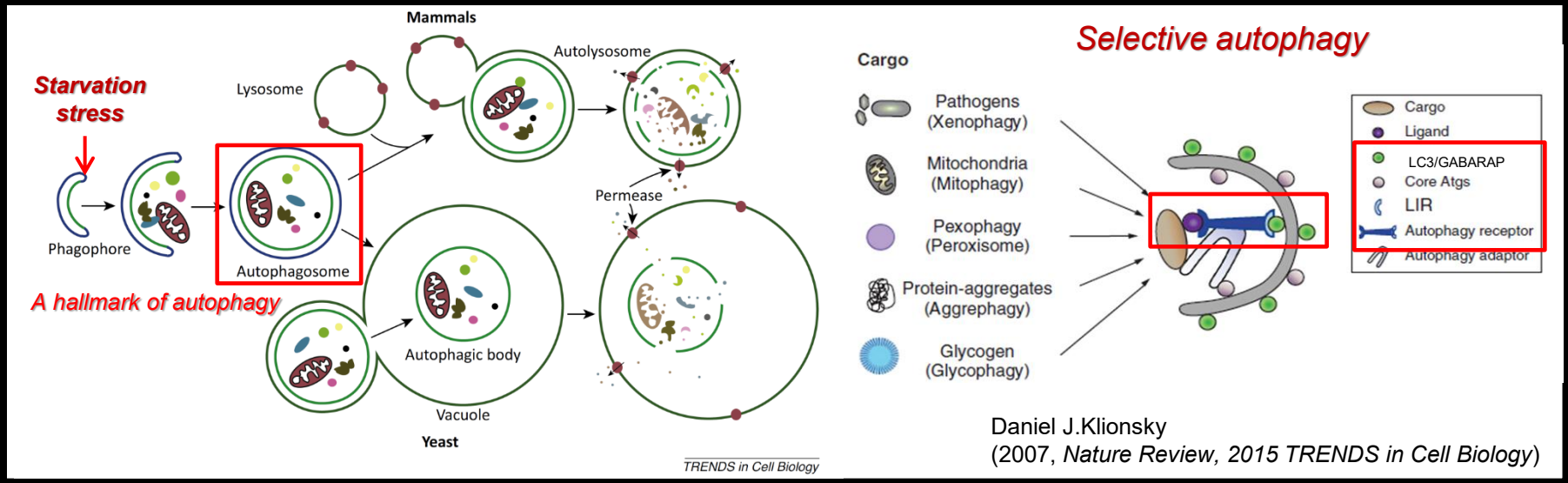


- Basal autophagy regulates cellular homeostasis in neurons
- Autophagy regulates synaptic development
- Basal and induced autophagy modulates presynaptic structure and synaptic transmission
- mTOR-dependent autophagy regulates synaptic pruning and autistic-like behaviors

Hara et al., 2016, Nature, Komatsu et al., 2006,Nature, Hernandez et al., 2012, Neuron, Tang et al., 2014, Neuron, Hui et al., 2019,Science Adv

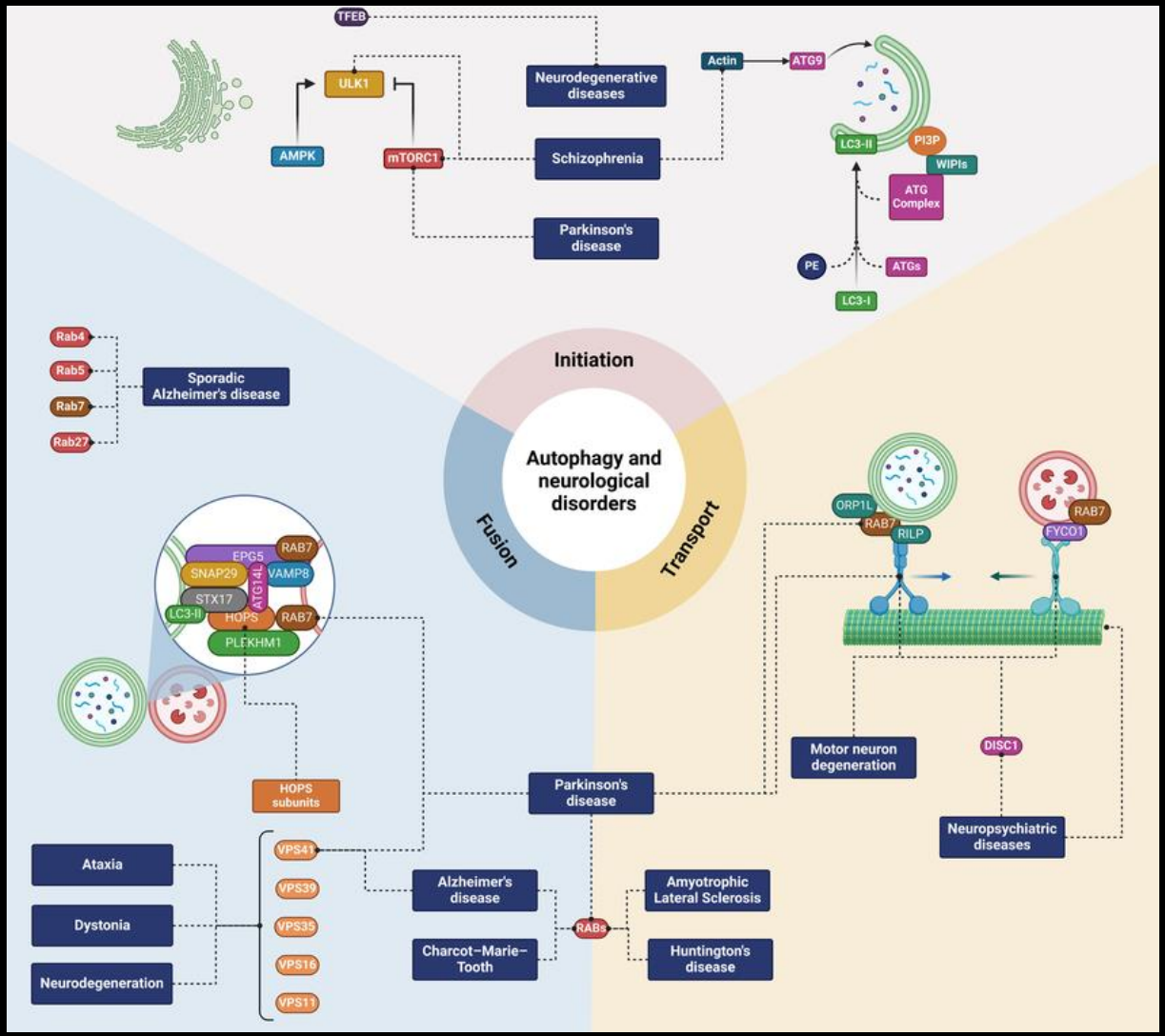
Selective Autophagy

- *A highly selective process* involving *recognition of specific cargoes* by *autophagy receptors*, which together *with adaptor proteins* link the cargo to the core autophagy machinery to allow its sequestration by autophagic membranes *via LC3/GABARAP-binding*



Daniel J.Klionsky
(2007, *Nature Review, 2015 TRENDS in Cell Biology*)

When Autophagy Falters-Link to Disease

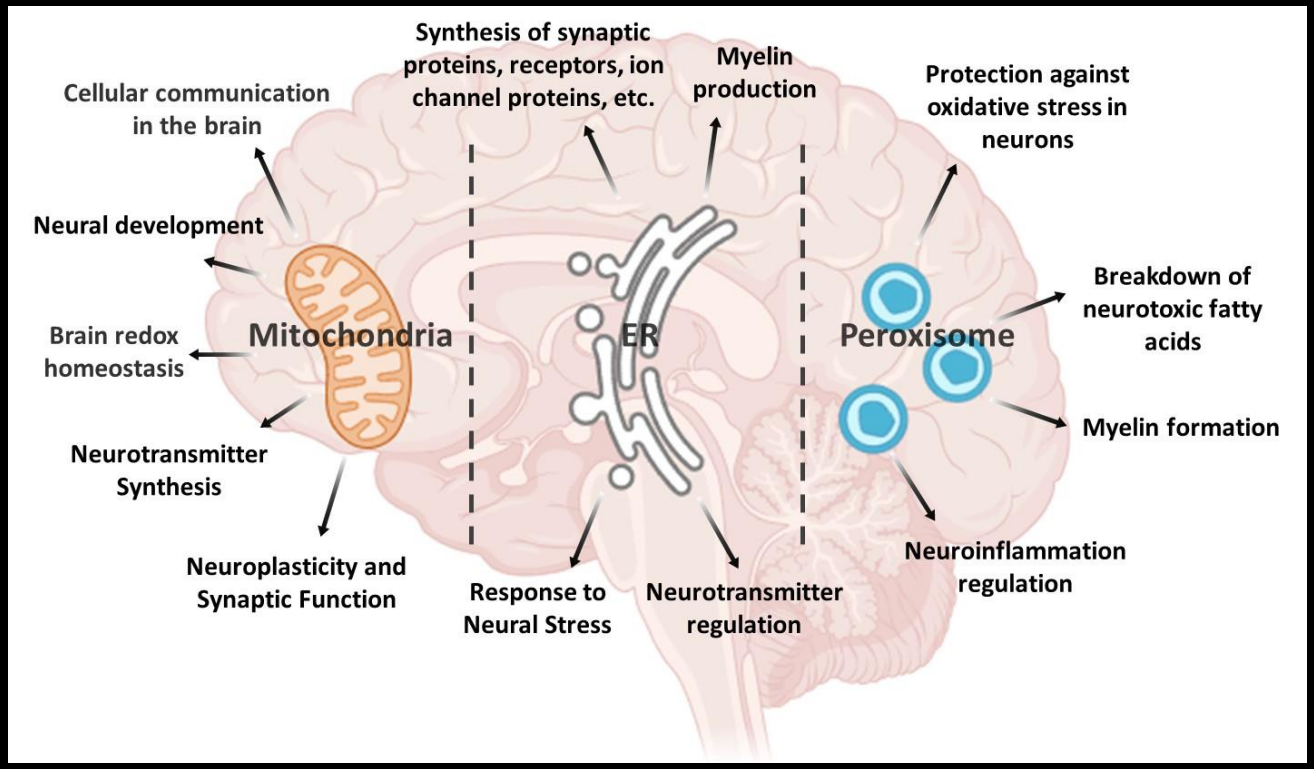


Silva et al., 2024, Journal of neurochemistry

Jina's Lab Research Overview

"Stress-selective autophagy- neurological disorder" axis

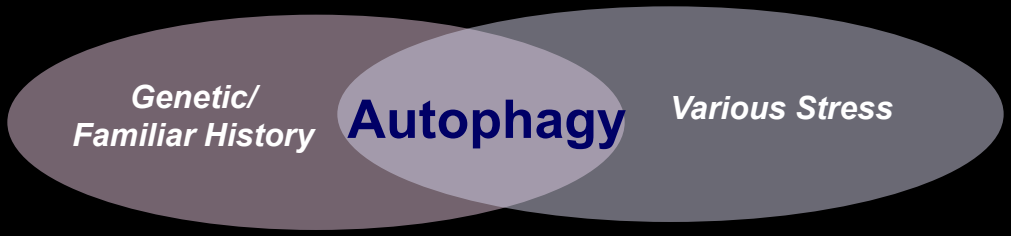
- *How do stress and impaired neuronal homeostasis drive neurological disorders?*
- *How do neurons handle damaged organelles in brain under stress?*



Main Research

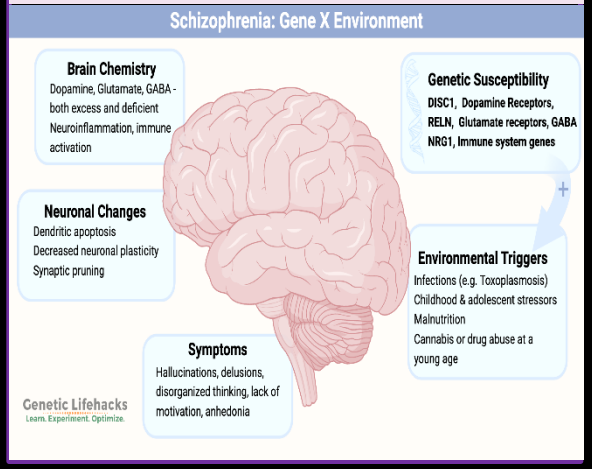
To identify molecular checkpoints at the intersection of stress and autophagy, and to restore neuronal homeostasis in stressed and diseased brains

Genetic factors **Environmental factors**

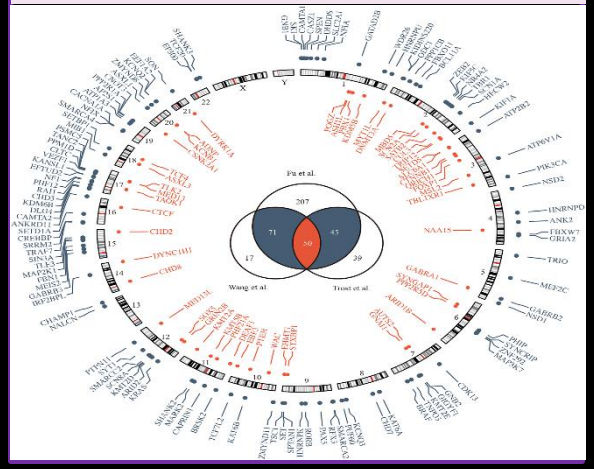


Neurological Disorder

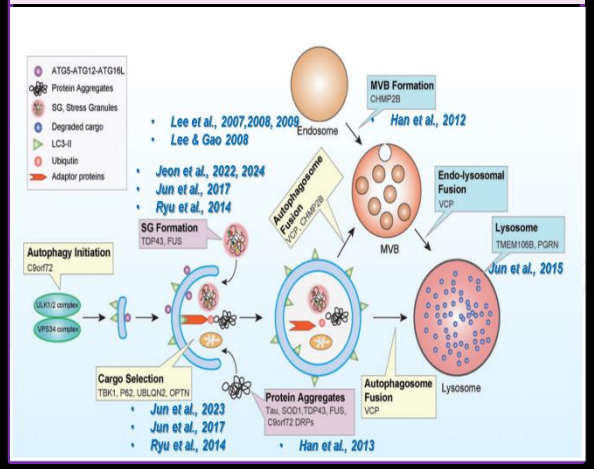
Autism/Schizophrenia



Rare neurodevelopmental disorders

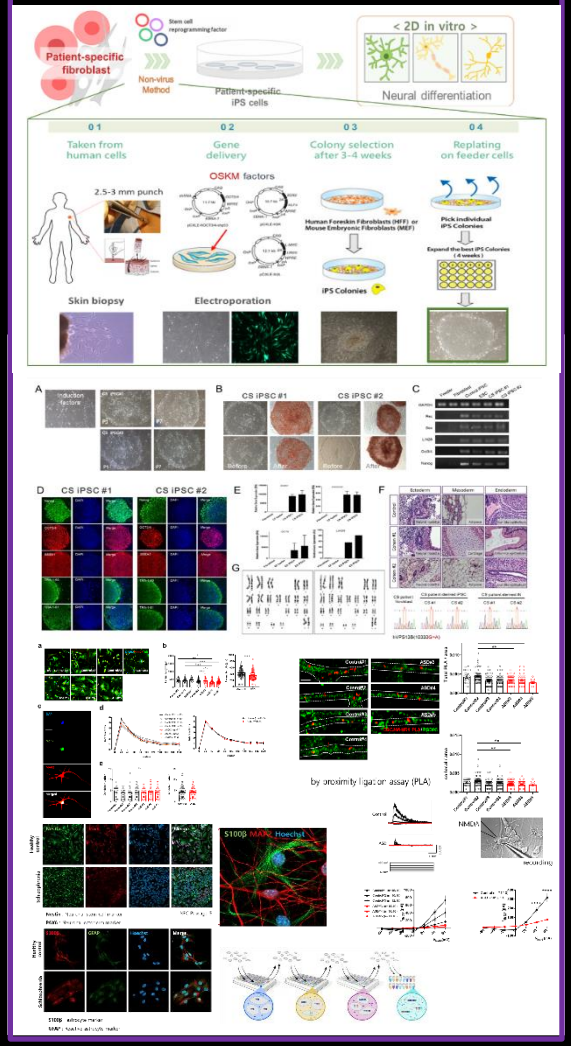


FTD/ALS

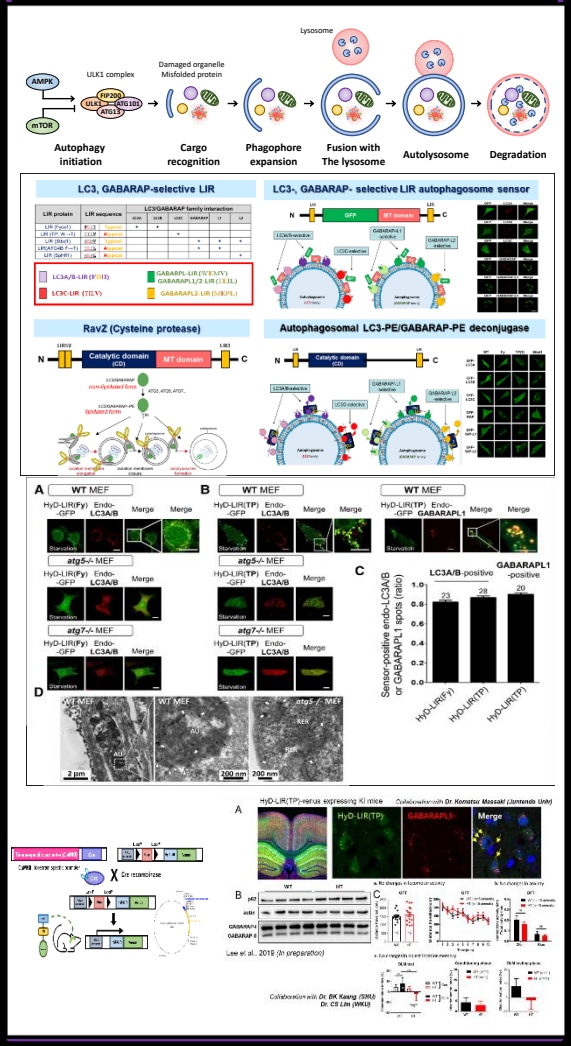


Main Research Tools

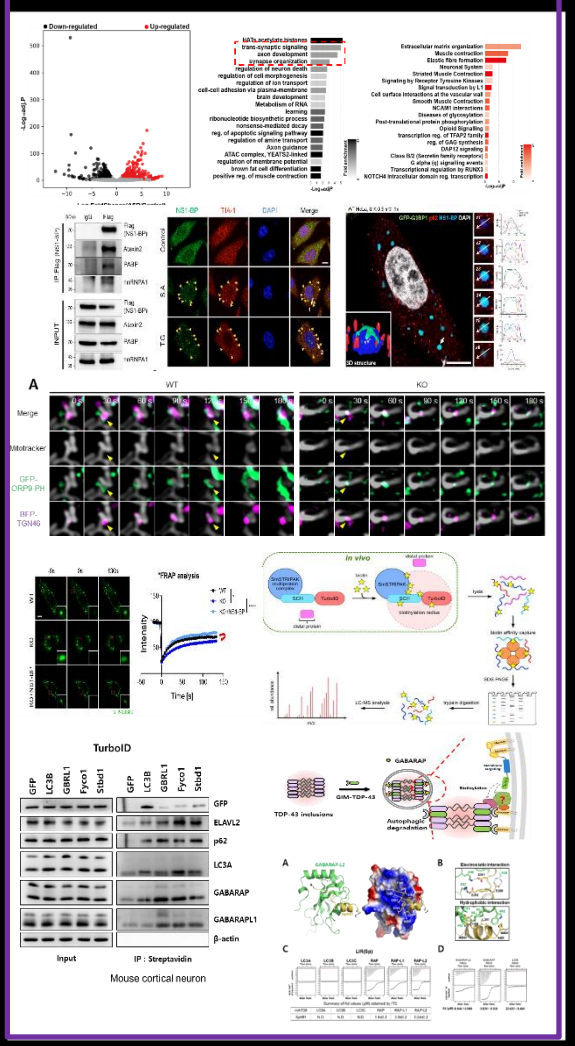
Disease Modeling by iPSC technology



Innovative Autophagy Tools & Autophagy Analysis



Molecular, Cellular & Biochemical Analysis

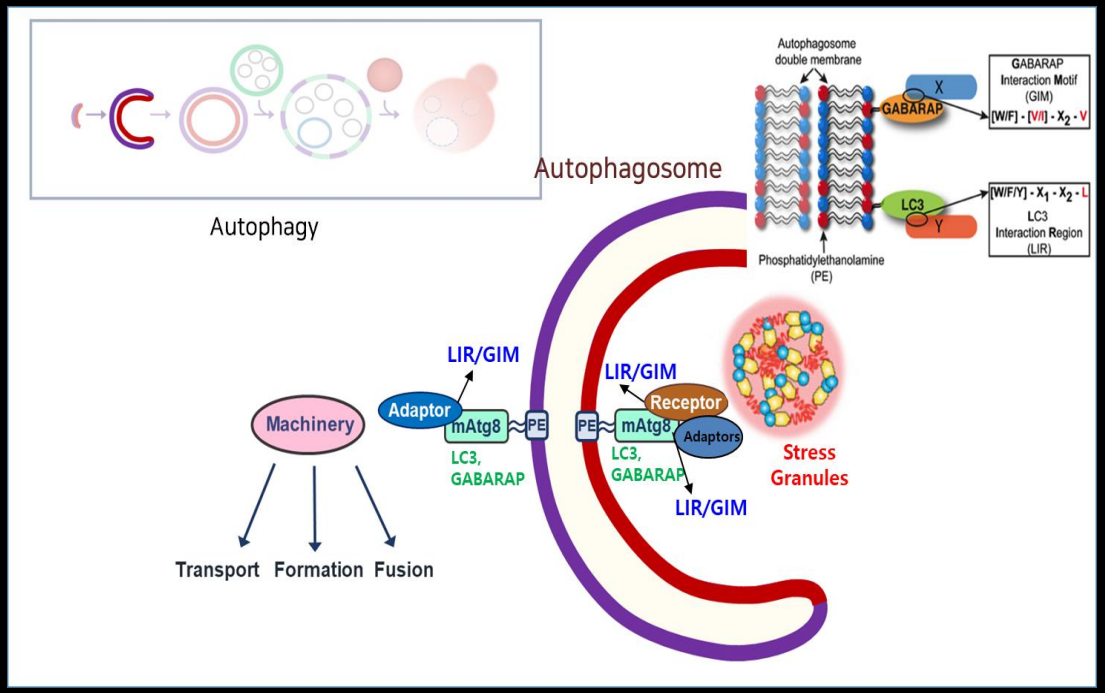


Current Research 1

Development of Innovative Tools for Autophagy Research

LC3/GABARAPs as Autophagy Markers

- LC3 and GABARAP proteins are specifically recruited to autophagosomal membranes during autophagy.
- During selective autophagy, LC3 or GABARAP proteins interact with selective autophagy receptors through short hydrophobic motifs called LIR or GIM, with varying affinities.



- Screening of LC3/GABARAP-interacting motifs (LIR/GIMs) that specifically bind to LC3 and GABARAP proteins

LIR protein	LIR motif seqeunce	LIR protein	LIR motif seqeunce
A16L1	EPANQTLKDEYQALQITFTALEGKL	NFATC4	SPGGRGPEDSWLLLSAPGPTPASPR
ADAMTS19	ELQFAPDREEWVVFPALWRREPVD	NFKBIL1	QKLQGELEDEWQVMGRFEGDASHE
ALG1	SSTSWTEDEDFSILLAALEKFEQLT	NS1-BP	GEMYDSNIDDWIFVPELRTNRCNAG
AMBRA1	EALNSGVEYIWQLNETVFTVHSNS	OFD1	ATEASSPESGFGFIASSTKAKVREL
APC	LRFKLESASFELLSPSRFASPTR	OSBPL6	PVPLSKEASGWIIEGLKIQGTNVQ
ATF4	AEESLGLLDDYLDVAKHFKPHGFSS	PCDH18	EIPENYEEDEFNVLNHLNDGKHEL
ATXN2	MESILFKCSDFVVQFKDMESSYAK	PEG3	IYECEDCGLGFVLTDLTTHQKVHS
Calreticulin-LIR2	QVESGSLEDEWFLPPKKIKDPDAS	PEX1	VEVEPLSADDWILELHAVSLEQHL
CCAR2	VWTIMPTLEEWALCQQKAAEAAPP	PICALM	STNVIVDSGGFVLGGLLKPTVASQ
Clathrin HC-LIR2	LNNLFITEEDYQALRTSIDAYDNFD	POLR2A	QSTVVAEDQEWVNVYYEMPDFDVAR
CSPG4	DPDAACEGLTFVVLDTSGLFVERR	PREPL	SKDEEADNDNYQVLFNLEELKLLQP
CWF19L2	DDTQIIKRDSWHTVIFNSVKTVSSS	PRMT1	WKQTVFYMEDYLVKTQEEIPGTIG
DDX3X	QMLARDFLDEYIFLAVGRVGSTSEN	PRMT2	LLQEGVQPEEFVAIADYAATDETQL
DNAAF4	FTLYKKEAAMWELSVTGVDKEMMQ	PRRC2A	VTEAIPVSREWELLPSAAASAEPQS
EGFR	ALTEDSIDDTFLPVPEYINQSVPKR	RANBP6	DVENMSDDDGWQFVNLGDQQSFGIK
EHMT2	TKGDPGSLEEWETVVGDDFSLYYDS	RhoA	ALWDTAGQEDYDRLRPLSYPDTDVI
EIF3A	DVMKSKKHRTWQNIHEPIMLKYLEL	RMDN2	LTSGLFEDEDFAVLFQDEDRSSPIE
FCGR2B	DPVHLTVLSEWLVLQTPHLEFQEGE	RUSC1	VPQAKKDRSDWLIVFSDDTELPPSG
FNIP2	LEKGEVKESKYVIFVRNEPALVPP	SF3A1	TIVPKPPPPEFEIADPPSISAFDL
FOXJ1	ELEPLKGNFIWAIFDAGTLGGELG	SHANK1	SHLPALTKEDYVLGVTRVGHRMNI
GAB1	APLEIKPLPEWELQAPVRSPITRS	SHANK2	PASFLPPPESFAVADSGIEEVDSR
GPSM1/AGS3	KRTQRLSAETWLLRLPLEREQNGD	SHANK3	AHLPALTKDDFVELGVTRVGHRMNI
HMGXB3	PPREVGEESEWEIVIISDAHVLVKE	SIPA1	EAGSGTLEDEWQAISEIASTCNTIL
HNRNPH1-p(S54,63E)	TREGRPEGEAFVSLEEDEVKLALK	SOX6	LVSTIQQDADWHSVLSSQQRMESEN
HNRNPU	EKPYFPIPEEYIIQNVPLEQKVRG	STK3	DNWKVPQDGDFQFLKNLSLEELQMR
Htt	SLGSFVHLPSYLLNDVLKATHANY	STK4	SLWKIPQDGDFQFLKSWTVDELQKR
IPO5	DMENMSDDDGWEFVNLGDQQSFGIK	SVIL	PRSPVEMDEDFVIFDPYAPKLTSS
LNPEP	YYIVHYADDEWEALIHQLKINPYVL	TBC1D15-LIR1	SEHLNSYEAEWIMVNTVSFKRKPHT
LRBA	SIVEEEEDDDYVELKVEGSPTEEAN	TBC1D15-LIR2	LKINQQEEPGFEVITRIDLGERPVV
MAK	GQTIFKSGDSWELEDYDFGASHSK	TBC1D2B	RRTVFYTNEEWELLDPTPKDLEESI
MAP15K	LSVPEYRSRVYQMILEQGGSSGTSR	TBC1D5-LIR3	GCTDRGNSDDFILISKDDDGSSARG
MAPK8IP1/JIP1(1)	HPSISEEEEGFCLSSPERAEPPGS	TNC	SIKETSVEVEWDELDIAFETWEIIF
MAPK8IP1/JIP1(2)	SLSSDTSALSYLVKYTLVVTHEHAQ	TUBA1C	REDMALEKDYEDVGADSADGEDEG
MCM2	ELIGDGMERDYRAIPELDAYEAEGL	ZNF862	DITVYLLQEEWVLLSQQQKELCGSN
MCM4	EALRALADDDFLTVTGKTVRLL	ScHfi1	EESWEDDIAGQRTFPEDPNYPV
MCM7	PAQFQAALDEYEELNVWQVNASRTR	SpHfi1	LQFEIDDEMEPLYNQAKQMRYGDY
METAP1	GWQLETWPDGWTAVTRDGKRSAQFE	SUPT6H	SFDDRLEDDDFGLIEENLGVKVKRG
MORF4L1	EELKPWLVDDWLITRQKQLFYLPA	SV2B-LIR1	LLEMGKHDEAWMILKQVHTNMRAK
MURF2B(TRIM55)	LLAFLILHYIWQIQCLIPTLMCWI	SV2B-LIR2	NIKTPFQMGEFLEIQSSTGTWYQEW
NALP13	EMMGALLETWNIGWPKIHVYIRN	TCPR2	YLTALTNGDYIAVGSSIGMLYLYC
NCOA4	AMTFSRIADSFVVIKNSPLSEWLIR	UBR4	QEQSEVDHGDFLMVSESMVLETAEN
NCOA7-LIR1	DLCPLYRPGEWELASEKDINPFSK	YWHAB	KNVVGARRSSWRVISSIEQKTERNE
NCOA7-LIR2	KENFLGEDDDFVDLEELSSQTGGGM	ZNF579	EEKQVLLQADWILLCLRCREAFATK
NEDD4-LIR2	SVDNQESSENWEIIREDEATMYSSQ	ZPR9	SEEEEEDLGDDWEDIDSDEELECEDT

Development of Probe and Deconjugase for Selective Autophagy

Motif identification and its application to probe and deconjugase development

LC3, GABARAP-selective LIR

LIR protein	LIR sequence		LC3/GABARAP family interaction					
			LC3A	LC3B	LC3C	GABARAP	L1	L2
LIR (Fyco1)	FDII	Typical	+	+				
LIR (TP, W→T)	TILV	Atypical			+			
LIR (Stbd1)	WEMV	Typical				+	+	+
LIR(ATG4B F→T)	TEIL	Atypical				+	+	
LIR (SpHfl1)	MEPL	Atypical						+

LC3A/B-LIR (FDII)

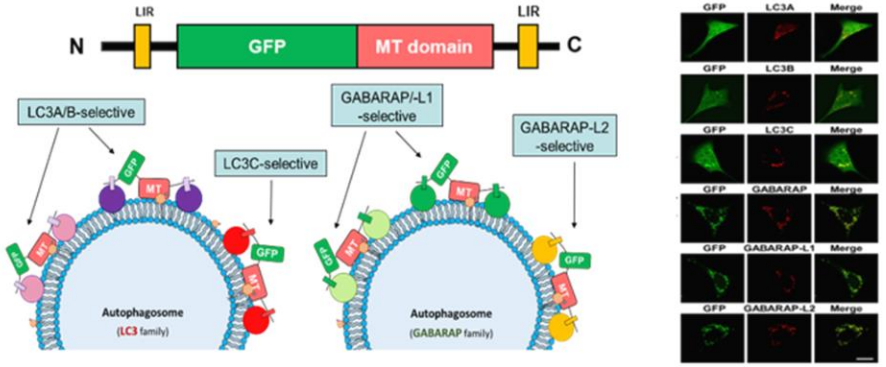
LC3C-LIR (TILV)

GABARPL-LIR (WEMV)

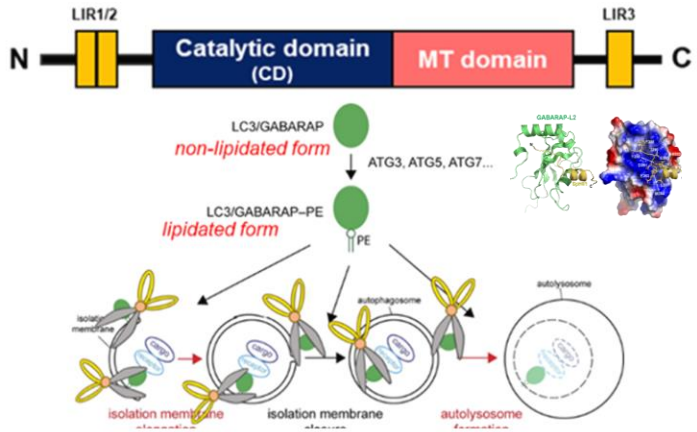
GABARAPL1/2-LIR (TEIL)

GABARAPL2-LIR (MEPL)

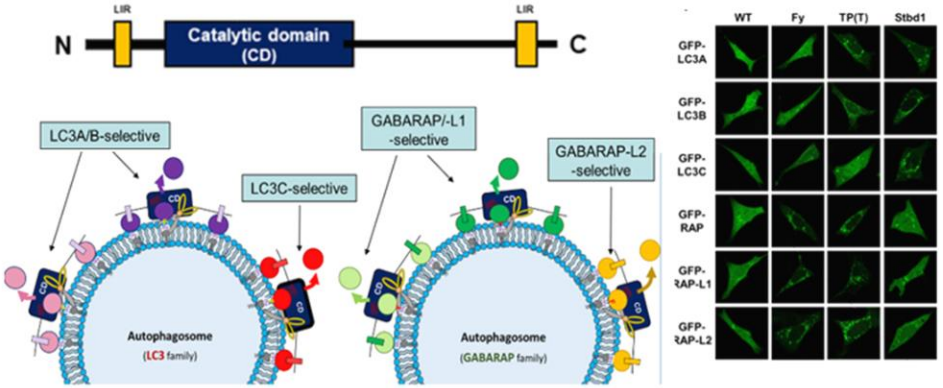
LC3-, GABARAP- selective LIR autophagosome sensor



RavZ (Cysteine protease)



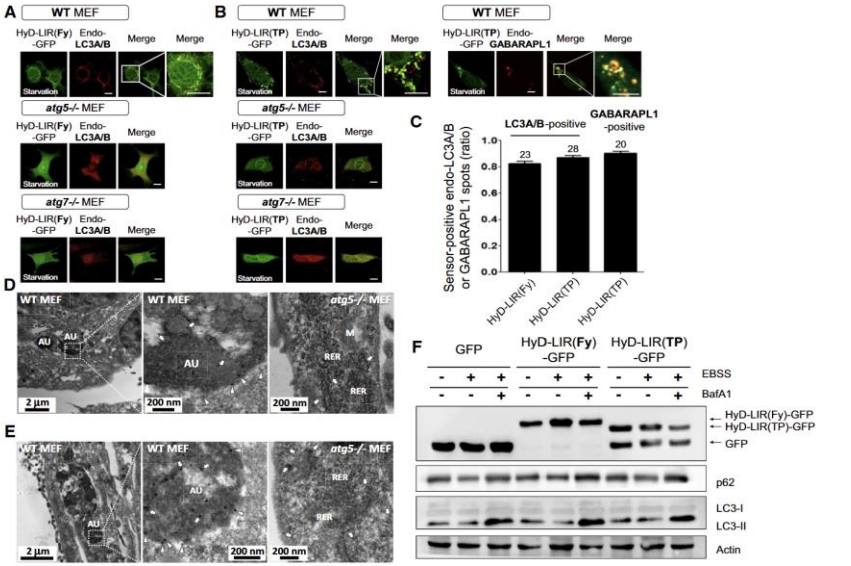
Autophagosomal LC3-PE/GABARAP-PE deconjugase



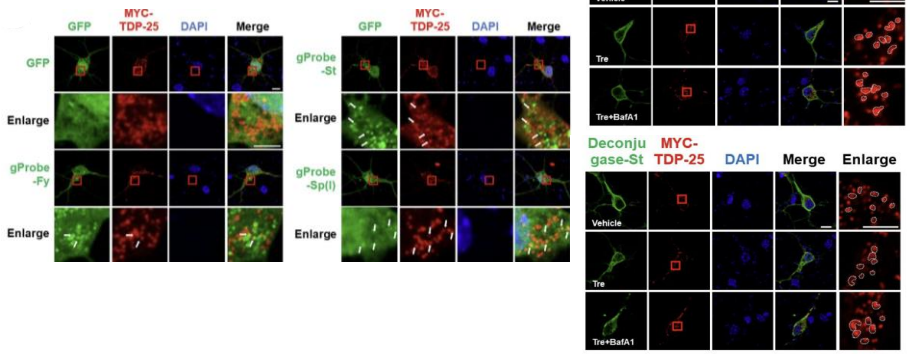
(Lee et al. 2017(EMBO J), Park et al.,2019(Sci. Rep), Park et al., 2023, 2024 (Autophagy))

Monitoring and Inhibition for Selective Autophagy (*In vitro/In vivo*)

- **Autophagy Monitoring *in vitro***

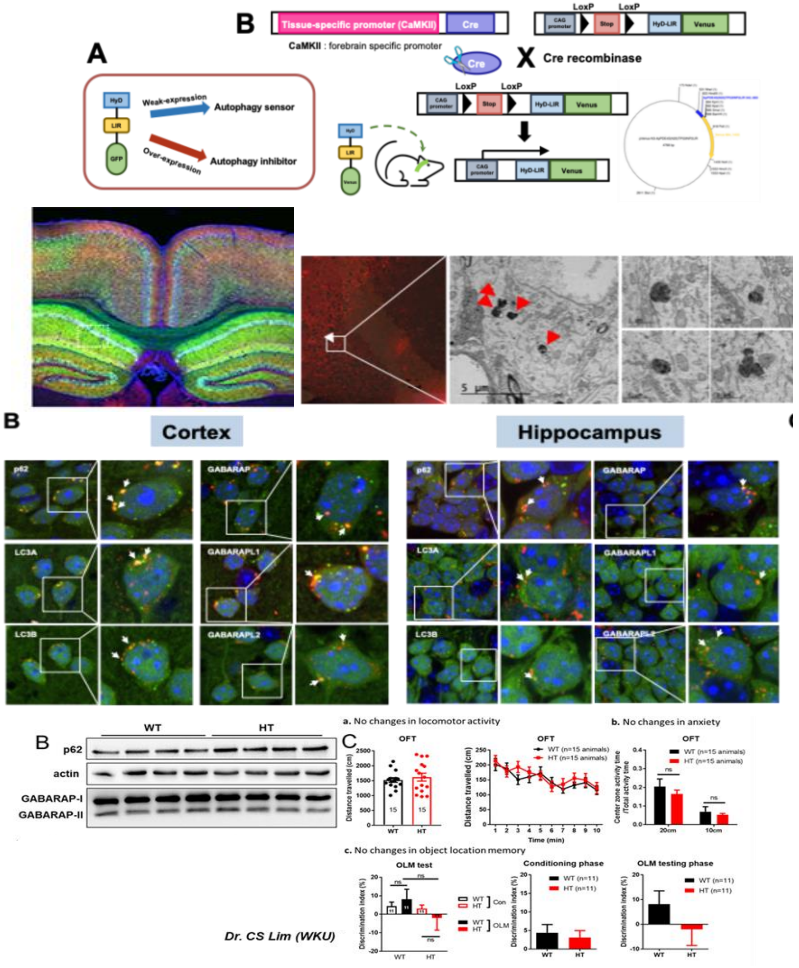


- **Autophagy Inhibition**



- **Autophagy Monitoring *in vivo***

CRE-inducible autophagy monitoring mice

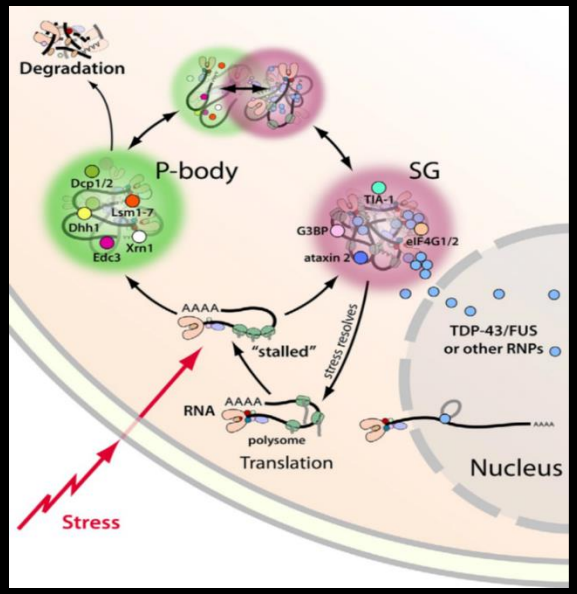


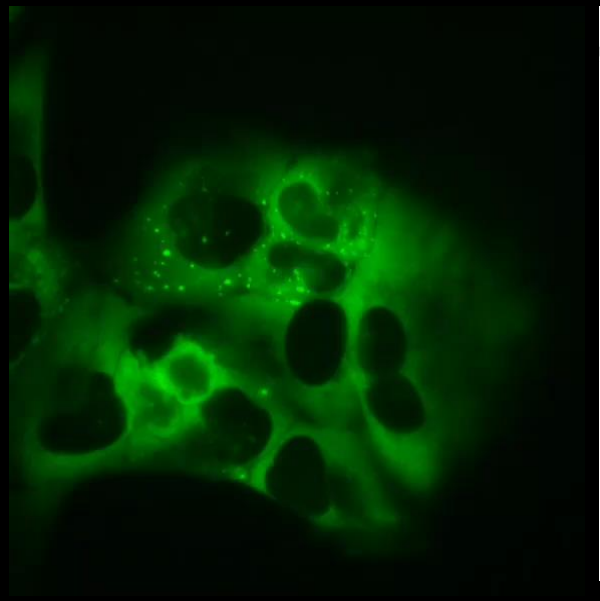
(Lee et al. 2017(EMBO J), Park et al.,2019(Sci. Rep), Park et al., 2023, 2024 (Autophagy))

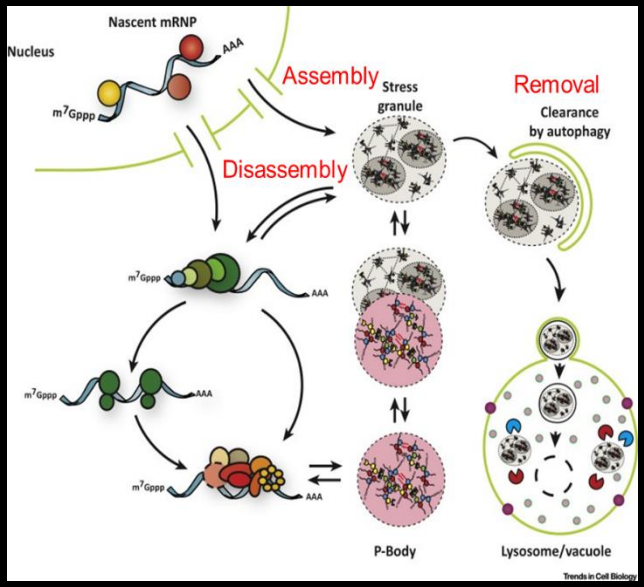
Current Research 2

Molecular Link between cellular stress, autophagy, and neurological disorders

Autophagy at the Crossroads of Stress and Disease

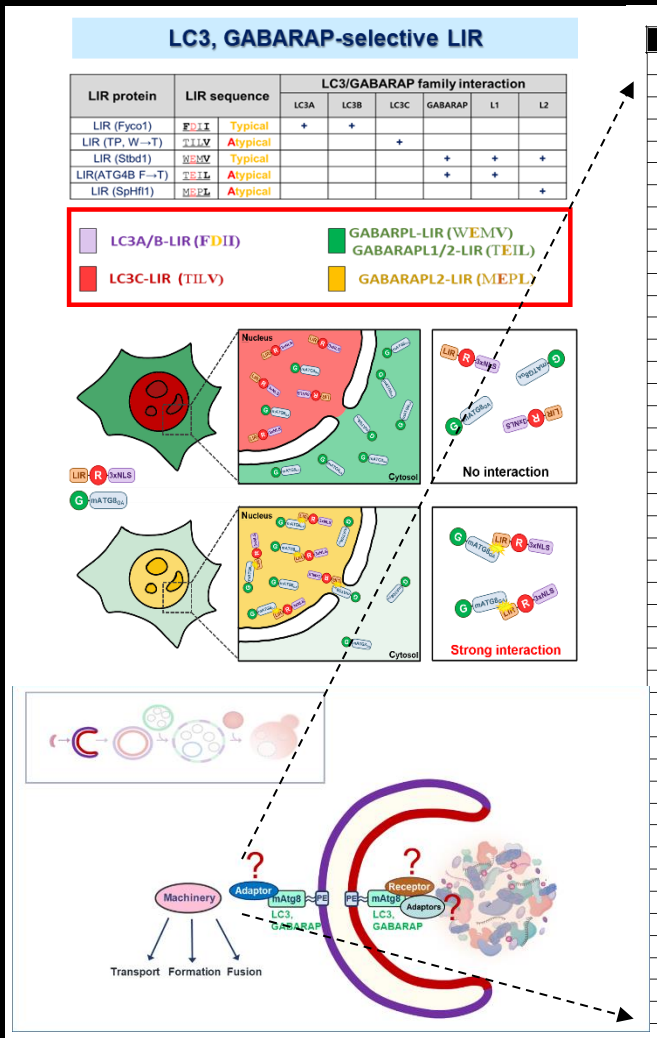






- Stress granules (SGs) are dynamic, cytoplasmic aggregates of proteins and RNA that form under stress to suppress translation initiation and help cells adapt.
- SGs serve as regulatory hubs for mRNA and protein homeostasis and are normally disassembled after stress resolution.
- Autophagy plays a critical role in clearing persistent or aberrant SGs; dysfunction in this process can contribute to neurological disorders.

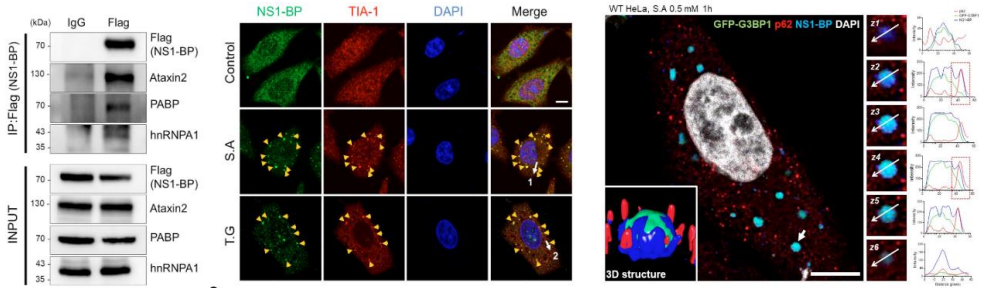
Potential candidates for selective autophagy receptors and adaptors: LIR/GIM-containing proteins



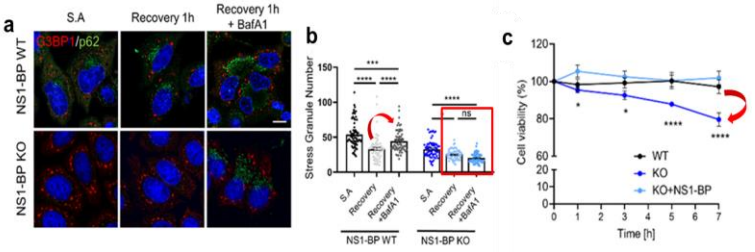
LIR protein	LIR motif seqeunce	LIR protein	LIR motif seqeunce
A16L1	ERANQTLKDEYDALQITFTALEGKL	NFATC4	SPGGRGPEDSWLLLSAPGPTPASPR
ADAMTS19	ELQFAPDREEWEVVFPALWRREPVD	NFKBIL1	QKLQGELEDEWQEVMGRFEGDASHE
ALG1	SSTSWTEDEDFSILLAALEKFEQLT	NS1-BP	GEMYDSNIDDWIPVPELRTNRCNAG
AMBRA1	EALNSGVEYYWDQLNETVFTVHSNS	OFD1	ATEASSPESDFEFIASSTKAKVREL
APC	LRRKLEESASFESLSPSSRPASPTR	OSBPL6	PVPLSKEADSWEIIEGLKIGQTNVQ
ATF4	AEESLGLLDDYLEVAKHFKPHGFSS	PCDH18	EIPENYEEDDFDNVLNHLNDGKHEL
ATXN2	MESILFKCSDFVVVQFKDMDSSYAK	PEG3	IYECEDCGLGFVDLTDLTDHQKVHS
Calreticulin-LIR2	QVESGSLEDDWDFLPPKKIKDPDAS	PEX1	VEVEPLSADDWEILELHAVSLEQHL
CCAR2	VWTIMPTLEEWEALCQQKAAEAAPP	PICALM	STNVIVDSGGFDELGGLLKPTVASQ
Clathrin HC-LIR2	LNNLFITEEDYQALRTSIDAYDNFD	POLR2A	QSTVVAEDQEWVNVYYEMPDFDVAR
CSPG4	DPDSACEGLTFQVLGTSSGLPVERR	PREPL	SKDEEADNDNYEVLFNLEELKLDQP
CWF19L2	DDTQIIKRDEWMTVDFMSVKTVSSS	PRMT1	WKQTVFYMEDYLTVKTGEEIFGTIG
DDX3X	QMLARDFLDEYIFLAVGRVGSTSEN	PRMT2	LLQEGVQPEEFVAIADYAATDETQL
DNAAF4	FTLYKKEAAMWETLSVTGVDKEMMQ	PRRC2A	VTEAIPVSRDWELLPSAAASAEPQS
EGFR	ALTEDSIDDTFLPVPEYINQSVPKR	RANBP6	DVENMSDDDGWQFVNLGDQQSFGIK
EHMT2	TKGDPGSLEEWETVVGDDFSLYYDS	RhoA	ALWDTAGQEDYDRLRPLSYPDTDVI
EIF3A	DVMKSKKHRTWQKIHEPIMLKYLEL	RMDN2	LTSGLFEDEDFAVLFQDEDRSSPIE
FCGR2B	DPVHLTVLSEWLVLQTPHLEFQEGE	RUSC1	VPQAKKDRSDWLIVFSPDTELPPSG
FNIP2	LEKGEVEESEYVVITVRNEPALVPP	SF3A1	TIVPKEPPPEFEFIADPPSISAFDL
FOXJ1	ELEPLKGNFDWEAIFDAGTLGGELG	SHANK1	SHLPALTKEDYVDLGVTRVGHRMNI
GAB1	APLEIKPLPEWEELQAPVRSPITRS	SHANK2	PASFLPPPESFDAVADSGIEEVDSR
GPSM1/AGS3	KRTQRLSAETWDLLRLPLEREQNGD	SHANK3	AHLPALTKDDFVELGVTRVGHRMNI
HMGXB3	PPREVGEESEWEEVIISDAHVLVKE	SIPA1	EAGSGTLEDEWQAISEIASTCNTIL
HNRNPH1-p(S54,63E)	TREGRPEGEAFVELEEEDEVKLALK	SOX6	LVSTIQQDADWDSVLSSQQRMESEN
HNRNPU	EKPYFPIPEEYTFIQNVPLEDRVRG	STK3	DNWKVPQDGDFDFLKNLSLEELQMR
Htt	SLGSFYHLPSYLKLHDVLKATHANY	STK4	SDWKIPQDGDYEFLKSWTVEDLQKR
IPO5	DMENMSDDDGWEFVNLGDQQSFGIK	SVIL	PRSPVEMDEDFDVIFDPYAPKLTSS
LNPEP	YYIVHYADDDWEALIHQLKINPYVL	TBC1D15-LIR1	SEHLNSYEAEWDMVNTVSFKRKPHT
LRBA	SIVEEEEDDDYVELKVEGSPTEEAN	TBC1D15-LIR2	LKINQQEEPGFEVITRIDLGERPVV
MAK	GQTIFKSGDSWEELEDYDFGASHSK	TBC1D2B	RRTVFYTNEEWELLDPTPKDLEESI
MAP15K	LSVPEYRSRVYQMILECGGSSGTSR	TBC1D5-LIR3	GCTDRGNSDDFILISKDDDGSSARG
MAPK8IP1/JIP1(1)	HPSISEEEEGFDCLSSPERAEPPGG	TNC	SIKETSVEVEWDPLDIAFETWEIIF
MAPK8IP1/JIP1(2)	SLSSDTSALSYDSVKYTLVVDEHAQ	TUBA1C	REDMAALEKDYEEVGADSADGEDEG
MCM2	ELIGDGMERDYRAIPELDAYEAEGL	ZNF862	DITVYLLQEEWVLLSQQQKELCGSN
MCM4	EALRALADDDFLTVTGKTVRLL	ScHfl1	EESWEDDIAGQRTFPEDPNYPV
MCM7	PAQFQAALDEYEELNVWQVNASRTR	SpHfl1	LQFEIDDEMEPLYNQAKQMRYGDY
METAP1	GWQDETWPDGWTAVTRDGKRSAQFE	SUPT6H	SFDDRLEDDDFDLIEENLGVKVKRG
MORF4L1	EELKPWLVDDWDLITRQKQLFYLPA	SV2B-LIR1	LLEMGKHDEAWMILKQVHDTNMRAK
MURF2B(TRIM55)	LLAFLILHYIWSQIQCLIFTLMDWI	SV2B-LIR2	NIKTPKQMDEFIEIQSSTGTWYQRW
NALP13	ENMKAELLETWDNISWPKDHVYIRN	TCPR2	YLTALDTNGDYIAVGSSIGMLYLYC
NCOA4	AMTPSRIADSFQVIKNSPLSEWLIR	UBR4	QEQSEVDHGDFEMVSESMVLETAEN
NCOA7-LIR1	DLCPLYRPGEWEDLASEKDINPFSK	YWHAB	KNVVGARRSSWRVISSIEQKTERNE
NCOA7-LIR2	KENFLGEDDDFVDLEELSSQTGGGM	ZNF579	EEKQVLLQADWTLLCLRCREAFATK
NEDD4-LIR2	SVDNQESSENWEIIREDEATMYSSQ	ZPR9	SEEEEEDLDGDDWEDIDSDEELECEDT

Identification of NS-BP1 as a Selective Autophagy Adaptor for SGs

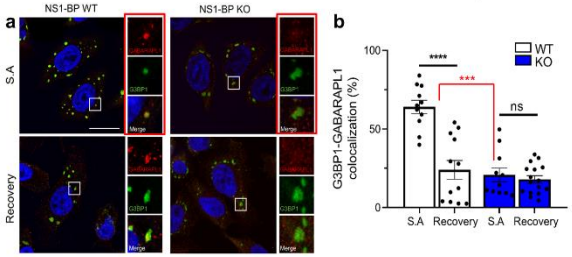
- **Interaction of NS-BP1 between GABARAP and p62**

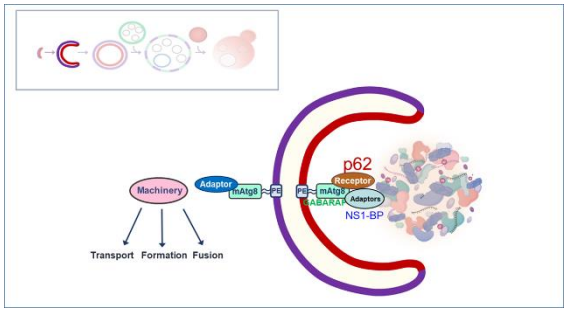


- **Impaired Autophagic Degradation of Stress Granules(SGs) in NS1-BP KO Cells**

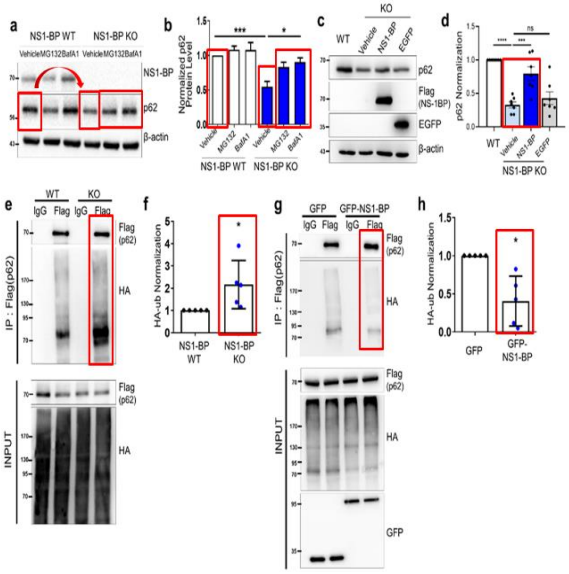


- **NS-BP1–mediated targeting of p62⁺ stress granules to GABARAP⁺ autophagosomes**

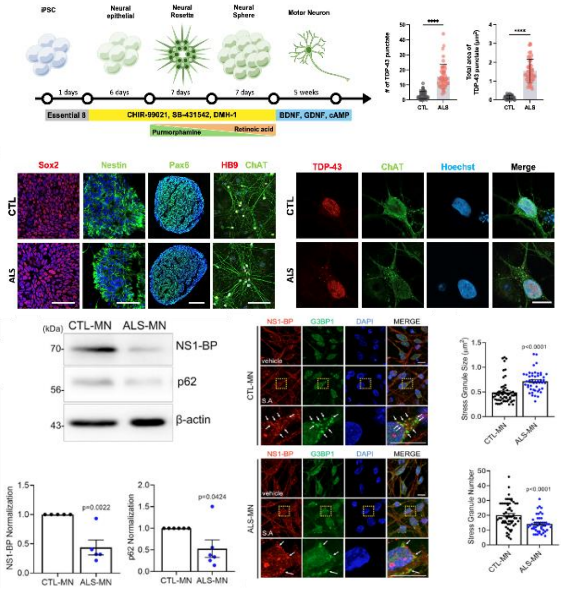




- **Negative Regulation of p62 Ubiquitination and Stability by NS1-BP**



- **Reduced Protein Level of NS-BP1 & Autophgic Degradation of SGs**



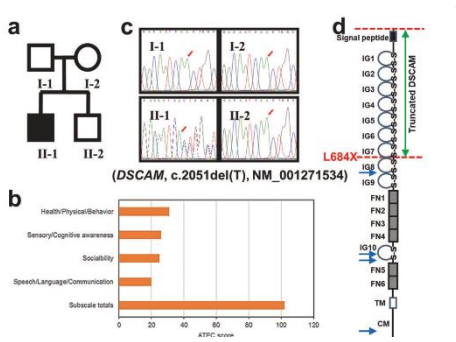
(Jeon et al., 2023 (Front Cell Dev Biol)), Jeon et al.,2024 (Nat. Commun.))

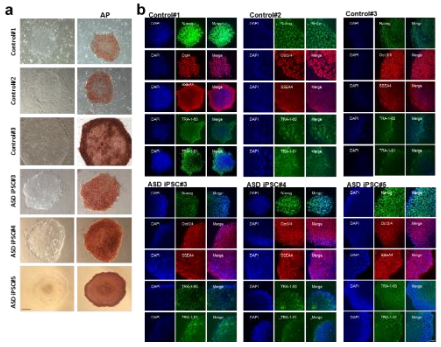
Current Research 3

Cellular Pathogenic Mechanism of Neurodevelopmental Disorders

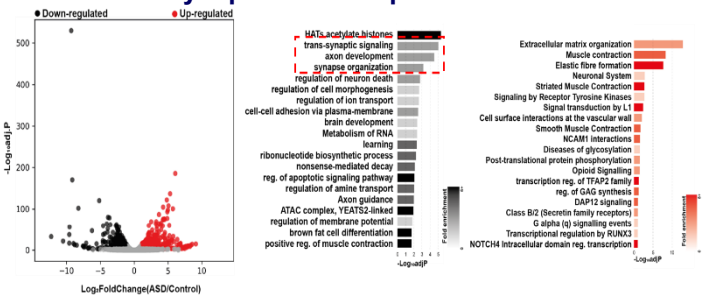
DSCAM Mutations in Autism Spectrum Disorders

- **Generation of iPSCs and Neuronal Differentiation from a Patient with a Heterozygous Missense Mutation in DSCAM**

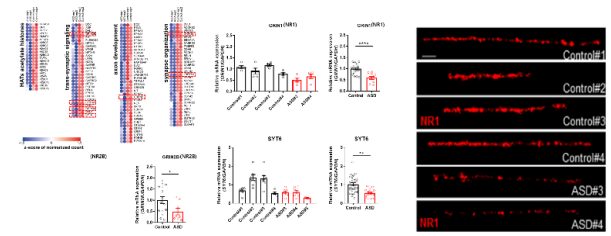


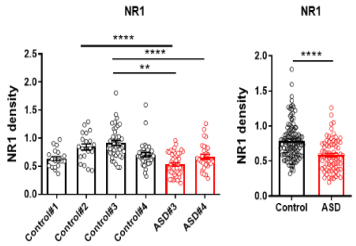


- **Aberrant Synaptic Gene Expression in ASD iN Cells**

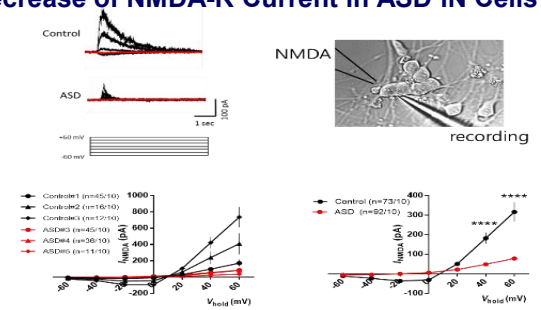


- **Decreased Expression of NR1 in ASD iN cells**

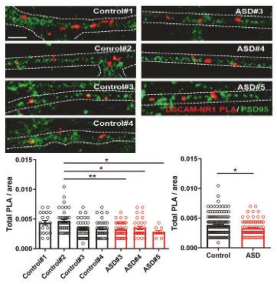


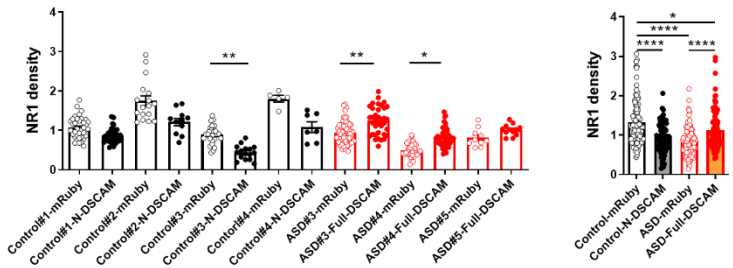


- **Decrease of NMDA-R Current in ASD iN Cells**

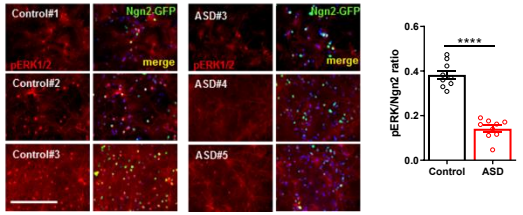


- **Interaction of DSCAM with NR1 in Dendritic Spines**





- **Reduced ERK Activation in ASD iN Cells**



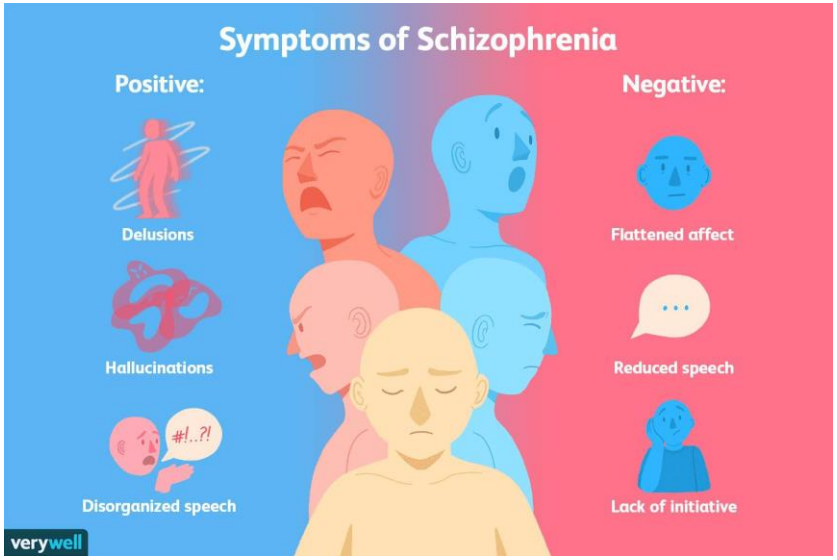
(Lim et al., 2021 (Mol. Psychiatry))

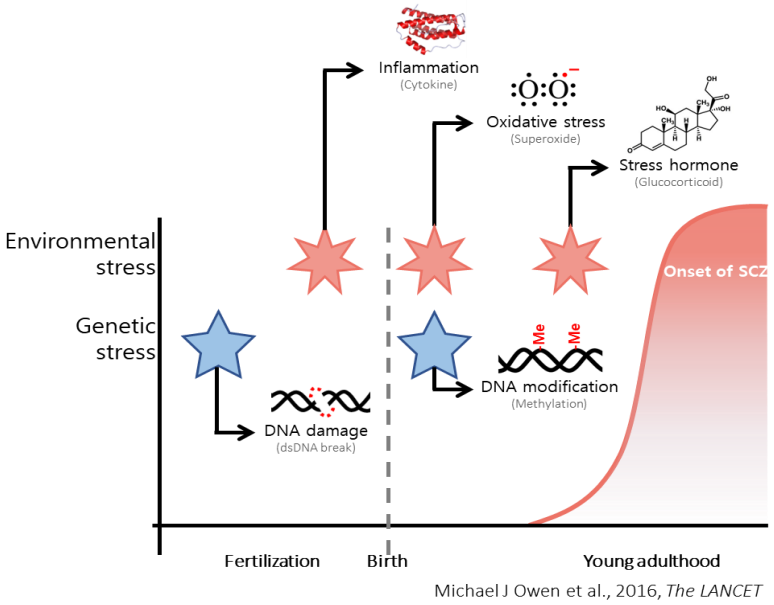
Ongoing Research

Topics to Explore Further in Neurodevelopmental Disorders

Mechanistic Insights into the Pathogenesis of Schizophrenia

- **Schizophrenia (SCZ)** is a chronic, disabling neuropsychiatric disorder affecting ~1% of the population, characterized by hallucinations, delusions, disorganized thought, and cognitive dysfunction.
- Despite advances in genetics and neuroimaging, the pathogenesis of SCZ remains elusive due to its clinical heterogeneity and the lack of human-relevant, cell-type–specific disease models.



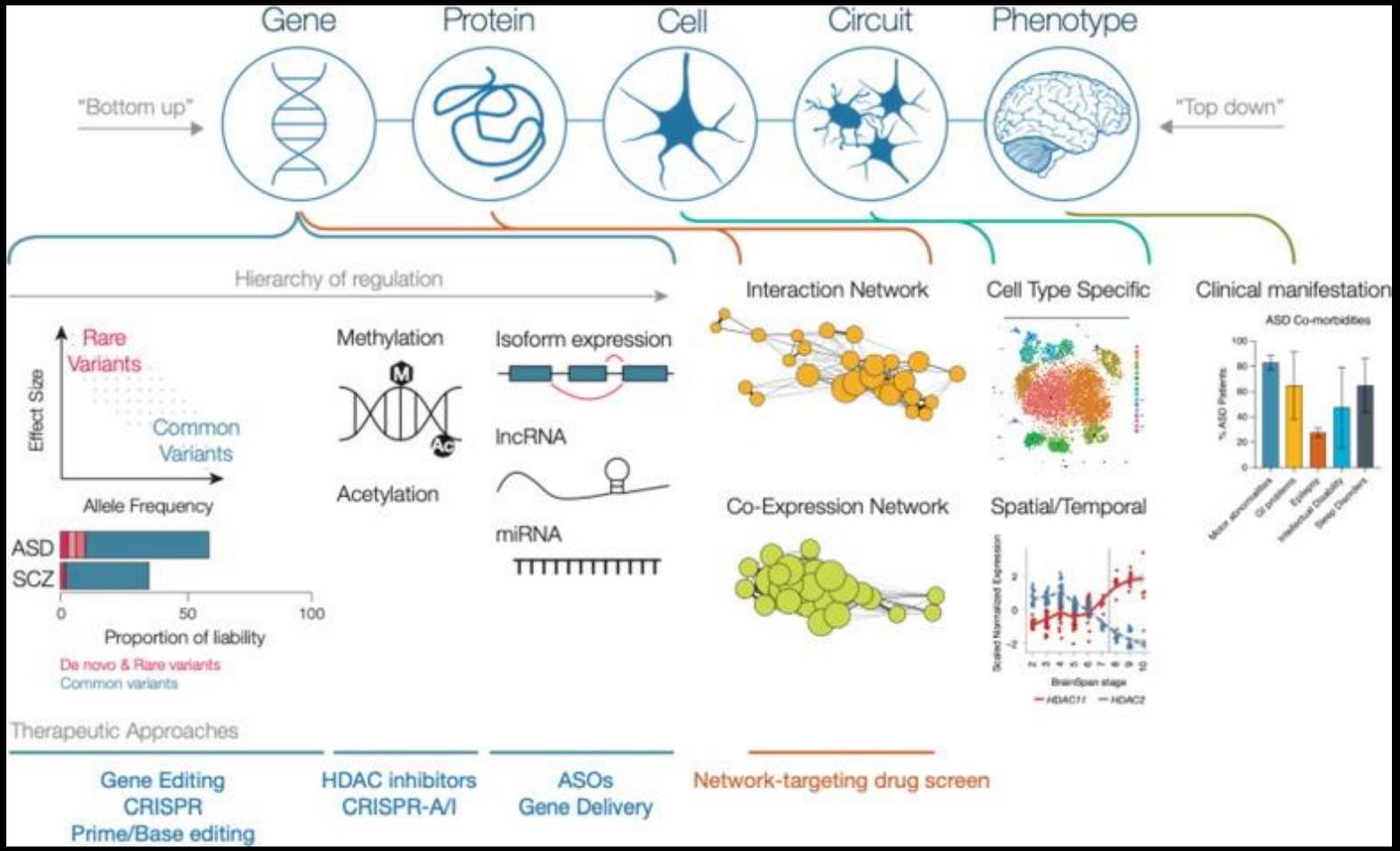


Michael J Owen et al., 2016, *The LANCET*

Open Questions

- How do neuron–astrocyte interactions shape disease-specific transcriptional landscapes?
- Can we stratify schizophrenia patients into molecular subtypes using cell-type–specific multi-omics?
- How does clozapine treatment influence neuron or astrocyte phenotype and transcriptome in treatment-resistant schizophrenia?
- What are the molecular mechanisms driving delayed neuronal maturation in schizophrenia iPSC-derived neurons?
- Are there clinically translatable biomarkers detectable from in vitro cellular models?

Interdisciplinary Research Using SCZ iPSC-derived Cells



Potential EU Collaboration Areas

- Integrative Cell-type Specific Multi-Omics
- Functional Studies in Neural Circuits or Behavioral Models
- Advanced Brain Organoid or Assembloid Systems for 3D Modeling of Cortical Development.
- Neurodevelopment and Psychiatric Mechanisms

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