Combinatorial selective ER-phagy remodels the ER during neurogenesis

Melissa Hoyer[1], Harper JW[1], Ian R. Smith[1], Julia C. Paoli[1], Yizhi Jiang[1], Joao A. Paulo[1]

[1]Harvard Medical School

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Harper

Melissa Hoyer

ABSTRACT

The endoplasmic reticulum (ER) has a vast proteomic landscape to perform many diverse functions including protein and lipid synthesis, calcium ion flux, and inter-organelle communication. The ER proteome is remodeled in part through membrane-embedded receptors linking ER to degradative autophagy machinery (selective ER-phagy). A refined tubular ER network is formed in neurons within highly polarized dendrites and axons. Autophagy-deficient neurons *in vivo* display axonal ER accumulation within synaptic ER boutons, and the ER-phagy receptor FAM134B has been genetically linked with human sensory and autonomic neuropathy. However, mechanisms, including receptor selectivity, that define ER remodeling by autophagy in neurons are limited**.** Here, we combine a genetically tractable induced neuron (iNeuron) system for monitoring extensive ER remodeling during differentiation with proteomic and computational tools to create a quantitative landscape of ER proteome remodeling via selective autophagy. Through analysis of single and combinatorial ER-phagy receptor mutants, we delineate the extent to which each receptor contributes to both magnitude and selectivity of ER clearance via autophagy for individual ER protein cargos. We define specific subsets of ER curvature-shaping proteins or lumenal proteins as preferred clients for distinct receptors. Using spatial sensors and flux reporters, we demonstrate receptor-specific autophagic capture of ER in axons, which correlates with aberrant ER accumulation in axons of ER-phagy receptor or autophagy-deficient neurons. This molecular inventory of ER proteome remodeling and versatile genetic toolkit provides a quantitative framework for understanding contributions of individual ER-phagy receptors for reshaping ER during cell state transitions.

MATERIALS

Protocol status: Working
We use this collection and it's working

Created: Jun 26, 2023

Last Modified: Aug 04, 2023

COLLECTION integer ID: 84033

Keywords: ASAPCRN, ER-phagy, induced neurons, axons, whole cell proteomics, differentiation, live cell fluorescence microscopy, Keima flux, autophagy

REAGENT or RESOURCE	SOURCE	IDENTIFIER	RRID
Antibodies			
FAM134B Rabbit Polyclonal Antibody	Proteintech	21537-1-AP	RRID:AB_2878879
FAM134C Rabbit Polyclonal Antibody	Sigma-Aldrich	HPA016492	RRID:AB_1853027
CCPG1 Rabbit Polyclonal Antibody	Cell Signaling Technology	80158	RRID:AB_2935809
TEX264 Rabbit Polyclonal Antibody	Sigma-Aldrich	HPA017739	RRID:AB_1857910
REEP1 Rabbit Polyclonal Antibody	Sigma-Aldrich	HPA058061	RRID:AB_2683591
REEP4 Rabbit Polyclonal Antibody	Sigma-Aldrich	HPA042683	RRID:AB_2571730
REEP5 Rabbit Polyclonal Antibody	Proteintech	14643-1-AP	RRID:AB_2178440
hFAB™ Rhodamine Anti-Tubulin Antibody	BioRad	12004166	RRID:AB_2884950
HSP90 mouse monoclonal Antibody	Proteintech	60318	RRID:AB_2881429
Anti-Keima-Red mAb	MBL international	M182-3M	RRID:AB_10794910
Neurofilament heavy polypeptide antibody	Abcam	ab7795	RRID:AB_306084
MAP2 Guinea Pig Polyclonal Antibody	Synaptic systems	188004	RRID:AB_2138181
Nogo-A (C-4) Mouse Monoclonal Antibody	Santa Cruz	sc-271878	RRID:AB_10709573
Calreticulin Rabbit Polyclonal Antibody	Proteintech	10292-1-AP	RRID:AB_513777
GAPDH (D16H11) XP Rabbit Monoclonal Antibody	Cell Signaling Technology	5174	RRID:AB_10622025
Goat anti-mouse Alexa488	Thermo Fisher Scientific	A-11001	RRID:AB_2534069
Goat anti-chicken Alexa488	Thermo Fisher Scientific	A11039	RRID:AB_2534096

Goat anti-rabbit Alexa568	Thermo Fisher Scientific	A-11011	RRID:AB_143157
Goat anti-rabbit Alexa647	Thermo Fisher Scientific	A27040	RRID:AB_2536101
Goat anti-guinea pig Alexa488	Thermo Fisher Scientific	**A-11073**	RRID:AB_2534117
Goat anti-guinea pig Alexa647	Thermo Fisher Scientific	A-21450	RRID:AB_141882
Bacterial and virus strains			
DH5 alpha E. coli competent cells	Homemade		
T1R E. coli Competent cells	Homemade		
Chemicals, peptides, and recombinant proteins			
DAPI	Thermo Fisher Scientific	D1306	
TMTpro™ 16plex Label Reagent Set	Thermo Scientific	A44520	
Q5 Hot Start High-Fidelity DNA Polymerase	New England BioLabs	M0493	
QuikChange II Site-Directed Mutagenesis Kit	Agilent	200523	
MiSeq Reagent Nano Kit v2 (300 cycles)	Illumina	MS-103-1001	
Bafilomycin A1	Cayman Chemical	88899-55-2	
DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride)	Thermo Fisher Scientific	D1306	
16% Paraformaldehyde, Electron-Microscopy Grade	Electron Microscopy Science	15710	
PhosSTOP	Sigma-Aldrich	T10282	
Protease inhibitor cocktail	Roche	4906845001	
TCEP	Gold Biotechnology	TCEP2	

Formic Acid	Sigma-Aldrich	94318	
Trypsin	Promega	V511C	
Lys-C	Wako Chemicals	129-02541	
Urea	Sigma	U5378	
EPPS	Sigma-Aldrich	E9502	
2-Chloroacetamide	Sigma-Aldrich	C0267	
Trypan Blue Stain Thermo Fisher Scientific	Wako Chemicals	129-02541w	
Bio-Rad Protein Assay Dye Reagent Concentrate	Bio-Rad	5000006	
Urea	Sigma	U5378	
EPPS	Sigma-Aldrich	E9502	
2-Chloroacetamide	Sigma-Aldrich	C0267	
Empore SPE Disks C18 3M	Sigma-Aldrich	66883-U	
Pierce Quantitative Colorimetric Peptide Assay	Thermo Fisher Scientific	23275	
GeneArt Precision gRNA Synthesis Kit	Thermo Fisher Scientific	A29377	
12 Well glass bottom plate with high performance #1.5 cover glass	Cellvis	P12-1.5H-N	
Nunc Cell-Culture Nunclon Delta Treated 6-well	Thermo Fisher Scientific	140685	
Nunc Cell-Culture Nunclon Delta Treated 12-well	Thermo Fisher Scientific	150628	
100x21mm Dish, Nunclon Delta	Thermo Fisher Scientific	172931	
Corning Matrigel Matrix, Growth Factor Reduced	Corning	354230	
DMEM/F12	Thermo Fisher Scientific	11330057	
Neurobasal	Thermo Fisher Scientific	21103049	
NEAA	Life Technologies	11140050	
GlutaMax	Life Technologies	35050061	
N-2 Supplement	Thermo Fisher Scientific	17502048	

Neurotrophin-3 (NT-3)	Peprotech	450-03	
Brain-derived neurotrophic factor (BDNF)	Peprotech	450-02	
B27	Thermo Fisher Scientific	17504001	
Y-27632 Dihydrochloride (ROCK inhibitor)	PeproTech	1293823	
Cultrex 3D Culture Matrix Laminin I	R&D Systems	3446-005-01	
Accutase	StemCell	7920	
FGF3	In-house	N/A	
Insulin Human	Sigma-Aldrich	I9278-5ML	
TGF-beta	PeproTech	100-21C	
holo-Transferrin human	Sigma-Aldrich	T0665	
Sodium Bicarbonate	Sigma-Aldrich	S5761-500G	
Sodium selenite	Sigma-Aldrich	S5261-10G	
Doxycycline	Sigma-Aldrich	D9891	
Recombinant SpCas9	Zuris et al., 2015; Orderu		
Hygromycin B	Thermo Fisher Scientific	10687010	
UltraPure 0.5M EDTA, pH 8.0	Thermo Fisher Scientific	15575020	
GlutaMAX	Thermo Fisher Scientific	35050061	
Dulbecco's MEM (DMEM), high glucose, pyruvate	GIBCO / Invitrogen	11995	
Lipofectamine 3000	Invitrogen	L3000008	
Experimental models: Cell lines			
HEK293T	ATCC	CRL-1573	CVCL_0045
H9	Wicell	WA9	CVCL_9773
Recombinant DNA			
pAC150-Keima-RAMP4	This paper		Addgene 201929
pAC150-Keima-REEP5	This paper		Addgene 201928
pAC150- FAM134C-GFP	This paper		Addgene 201932

	pAC150- TEX264-GFP	This paper		Addgene 201931
	pAC150-TEX264(deltaLIR, F273A)-GFP	This paper		Addgene 201930
	pHAGE-FAM134C-GFP	This paper		Addgene 201927
	pHAGE-TEX264-GFP	An et al 2019		Addgene 201925
	pHAGE-TEX264(deltaLIR,F273A)-GFP	An et al 2019		Addgene 201926
	pHAGE-mCherry-LC3B	An et al 2019		Addgene 201924
	Software and algorithms			
	Prism	GraphPad, V9	https://www.graphpad.com/scientificsoftware/prism/	SCR_002798
	SEQUEST	Eng et al., 1994	N/A	
	Flowjo	Flowjo, v10.7	https://www.flowjo.com	SCR_008520
	Perseus	Perseus v1.6.15.0 Tyanova et al. (2016)	https://maxquant.org/perseus/	SCR_007358
	Fiji	ImageJ V.2.0.0	https://imagej.net/software/fiji/	SCR_002285
	Imagelab	Biorad, v6.0.1	https://www.bio-rad.com/en-us/product/image-lab-software?ID=KRE6P5E8Z&source_wt=imagelabsoftware_surl	SCR_014210
	Cell Profiler	CellProfiler v4.0.6	https://cellprofiler.org/	SCR_007358
	Nikon Imaging Software Elements	5.21.3 (Build 1489)		SCR_014329

	outknocker.org	http://www.outknocker.org/outknocker2.htm		
	ChopChop	https://chopchop.cbu.uib.no/		SCR_015723
	Instruments			
	Orbitrap Fusion Lumos Tribrid Mass Spectrometer	Thermo Fisher Scientific	IQLAAEGAAPFADBMBHQ	CR_020562
	Orbitrap Eclipse Tribrid Mass Spectrometer	Thermo Fisher Scientific	FSN04-10000	SCR_020559
	Attune NxT	Thermo Fisher Scientific		SCR_019590
	Sony Biotechnology SH800S Cell Sorter	Sony Biotechnology	SH800S	SCR_018066
	Neon™ Transfection System	Thermo Fisher Scientific	MPK5000	N/A
	ChemiDoc MP imaging system	BioRad	12003154	SCR_019037
	Yokogawa CSU-X1 spinning disk confocal on a Nikon Ti-E inverted microscope	Yokogawa/ Nikon		

FILES

Protocol

NAME

Characterizing spatial and temporal properties of ER-phagy receptors

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Analysis of ER structures in Cultured Induced Neuron axons

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Analysis of ER Flux in Cultured Induced Neurons using Keima ER reporters

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Electroporation of Cas9 protein into human pluripotent stem cells

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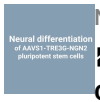

NAME

Neural differentiation of AAVS1-TRE3G-NGN2 pluripotent stem cells

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Whole-cell proteomics and Analysis by Tandem Mass Tagging-based proteomics

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