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Combinatorial selective ER-phagy remodels the ER during neurogenesis V.2

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Keywords: ASAPCRN, ER-phagy, induced neurons, axons, whole cell proteomics, differentiation, live cell fluorescence microscopy, Keima flux, autophagy, er during neurogenesis, axonal er accumulation within synaptic bouton, er shaping protein, quantitative landscape of er proteome remodeling, tubular er membranes within autophagosome, er during cell state transition, axonal er accumulation, molecular inventory of er, endoplasmic reticulum, specific autophagic capture of er, er proteome remodeling, neurogenesis, selectivity of er protein clearance, specific subsets of er membrane, er protein clearance, er membrane, process of neurogenesis, phagy receptor fam134b, refined tubular er network, diverse proteome landscape, role for autophagy, formed neuronal projection, selective autophagy, deficient neuron, synaptic bouton, deficient neurons in vivo, tubular er membrane, autophagosome, neuronal projection, neuron, er proteome, phagy receptor mutant, phagy receptor, autophagy, selective removal of er, embedded er, lumenal protein, tracta

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Abstract

The endoplasmic reticulum (ER) employs a diverse proteome landscape to orchestrate many cellular functions ranging from protein and lipid synthesis to calcium ion flux and inter-organelle communication. A case in point concerns the process of neurogenesis: a refined tubular ER network is assembled via ER shaping proteins into the newly formed neuronal projections to create highly polarized dendrites and axons. Previous studies have suggested a role for autophagy in ER remodeling, as autophagy-deficient neurons *in vivo* display axonal ER accumulation within synaptic boutons, and the membrane-embedded ER-phagy receptor FAM134B has been genetically linked with human sensory and autonomic neuropathy. However, our understanding of the mechanisms underlying selective removal of ER and the role of individual ER-phagy receptors is limited. Here, we combine a genetically tractable induced neuron (iNeuron) system for monitoring ER remodeling during *in vitro* 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 protein clearance. We define specific subsets of ER membrane 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, and directly visualize tubular ER membranes within autophagosomes in neuronal projections by cryo-electron tomography. 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

	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	
	100×21mm 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	3505006 1	
	Dulbecco's MEM (DMEM), high glucose, pyruvate	GIBCO / Invitrogen	11995	
	Lipofectamine 3000	Invitrogen	L300000 8	
	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://w	SCR_002798



			www.graphpad.com/scientificsoftware/prism/	
	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.biorad.com/en-us/product/image-lab-software?ID=KRE6P5E8Z&source_wt=imagelabsoftware_url	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

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