

ATG5 Overexpression is Neuroprotective and Attenuates Cytoskeletal and Vesicle-Trafficking Alterations in Axotomized Motoneurons

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SUPPLEMENTARY INFORMATION

- **Supplementary Tables and Headings**
- **Supplementary Figures and Legends**

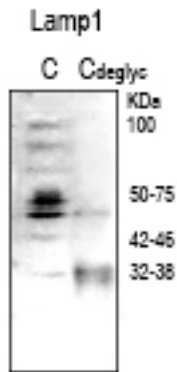
SUPPLEMENTARY TABLES AND HEADINGS

Supplementary table S1

Table S1. Fold change of microtubule and vesicle related proteins by proteomics							
GO:0015630 Microtubule cytoskeleton							
ID	Protein	Short Name	Fold	Std Error	t value	DF	p value
Q7TP36	Protein Shroom2	SHRM2	-3,04	0,11	-27,97	56	0
P34067	Proteasome subunit beta type-4	PSB4	-1,16	0,14	-8,55	137	4,64E-13
Q63560	Microtubule-associated protein 6	MAP6	-1,00	0,06	-17,85	280	0
P63004	Platelet-activating factor acetylhydrolase IB subunit alpha	LS1	-0,56	0,07	-8,03	416	2,27E-13
Q9Z1E1	Flotillin-1	FLOT1	-0,51	0,09	-5,49	8	0,00298825
Q7M6Z5	Kinesin-like protein KIF27	KIF27	-0,45	0,10	-4,53	14	0,00252115
P23928	Alpha-crystallin B chain	CRYAB	-0,45	0,11	-3,88	111	0,00108087
B08NF1	Septin-8	SEPTIN8	-0,40	0,08	-4,95	37	0,0001273
Q9WVC0	Septin-7	SEPTIN7	-0,39	0,09	-4,21	164	0,000232
P56536	Kinesin heavy chain isoform 5C	KIF5C	-0,39	0,08	-4,96	15	0,00104572
P62161	Calmodulin OS-Rattus norvegicus	CALM	-0,32	0,09	-3,52	247	0,00263141
P85515	Alpha-centractin	ACTZ	-0,31	0,12	-2,48	203	0,04239646
P38650	Cytoplasmic dynein 1 heavy chain 1	DYHC1	-0,28	0,03	-9,62	2339	0
O55156	CAP-Gly domain-containing linker protein 2	CLIP2	-0,18	0,05	-3,50	132	0,00317932
Q9QXU8	Cytoplasmic dynein 1 light intermediate chain 1	DC1L1	-0,17	0,06	-2,84	175	0,01821581
P63331	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	PP2AA	0,16	0,05	3,28	16	0,01719956
Q3KRE8	Tubulin beta-2B chain	TBB2B	0,25	0,08	3,22	16	0,01915378
Q04631	protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha	FNTA	0,26	0,08	3,39	118	0,00451095
Q02293	Protein farnesyltransferase subunit beta	FNTB	0,34	0,13	2,72	22	0,03921408
Q62812	Myosin-9	MYH9	0,68	0,12	5,87	98	7,03E-07
P31577	Ezrin	EZR1	2,37	0,26	9,25	8	0,0001186
P37397	Calponin-3	CNN3	3,35	0,17	19,63	36	0
Q6AYZ1	Tubulin alpha-1C chain	TBA1C	9,33	0,28	32,95	12	7,87E-12
GO:0031988 Membrane-bounded vesicle							
ID	Protein	Short Name	Fold	Std Error	t value	DF	p value
Q9Z216	Synaptic vesicle glycoprotein 2C	SV2C	-0,66	0,25	-2,58	47	0,04016482
P02770	Serum albumin OS-Rattus norvegicus	ALBU	-0,58	0,06	-9,56	548	0
Q63377	Sodium/potassium-transporting ATPase subunit beta-3	AT1B3	-0,57	0,21	-2,73	56	0,02829316
P12785	Fatty acid synthase	FAS	-0,55	0,05	-12,24	1042	0
P06685	Sodium/potassium-transporting ATPase subunit alpha-1	AT1A1	-0,53	0,07	-7,22	312	7,34E-11
Q9Z1E1	Flotillin-1 OS-Rattus norvegicus	FLOT1	-0,51	0,09	-5,49	8	0,00298825
P08082	Clathrin light chain B OS-Rattus norvegicus	CLCB	-0,46	0,06	-7,63	16	9,84E-06
Q9IKE3	Secretory carrier-associated membrane protein 5	SCAM5	-0,45	0,10	-4,77	16	0,00126997
P62824	Ras-related protein Rab-3C	RAB3C	-0,45	0,13	-3,51	53	0,00443394
Q05683	Glutamate decarboxylase 2	DCE2	-0,44	0,18	-2,51	63	0,04413076
P10824	Guanine nucleotide-binding protein (G(i)) subunit alpha-1	GNAI1	-0,39	0,06	-6,91	38	4,02E-07
P11598	Protein disulfide-isomerase A3	PDI3A	-0,38	0,06	-5,93	378	8,64E-08
P29101	Synaptotagmin-2	SYT2	-0,36	0,10	-3,65	237	0,00179161
Q9QVF3	Unconventional myosin-Va	MYO5A	-0,35	0,07	-5,35	172	2,89E-06
P08413	Calcium/calmodulin-dependent protein kinase type II subunit beta	Camk2B	-0,34	0,10	-3,44	86	0,00436509
Q63941	Ras-related protein Rab-3B	RAB3B	-0,33	0,11	-3,04	31	0,01743794
P21707	Synaptotagmin-1	SYT1	-0,33	0,08	-4,24	120	0,00030561
Q04639	Apolipoprotein A-I	APOA1	-0,32	0,09	-3,68	51	0,00286613
P6P6R2	Dihydropyridyl dehydrogenase, mitochondrial	DLDH	-0,30	0,07	-4,22	223	0,00025467
P63012	Ras-related protein Rab-3A	RAB3A	-0,29	0,09	-3,05	142	0,01117429
P05712	Ras-related protein Rab-2A	RAB2A	-0,27	0,06	-4,48	190	0,00010494
Q6AXT5	Ras-related protein Rab-21	RAB21	-0,25	0,10	-2,49	51	0,04811081
P13233	2',3'-cyclic-nucleotide 3'-phosphodiesterase	CN37	-0,25	0,06	-3,96	559	0,0005567
P11442	Clathrin heavy chain 1	CLH	-0,25	0,04	-6,46	1787	2,10E-09
P62815	V-type proton ATPase subunit B, brain isoform	VATB2	-0,23	0,07	-3,05	639	0,01005739
Q9QXU9	ProSAAS	PCSK1	-0,20	0,05	-4,12	105	0,0004988
O55156	CAP-Gly domain-containing linker protein 2	CLIP2	-0,18	0,05	-3,50	132	0,00317932
P63039	60 kDa heat shock protein, mitochondrial	CH60	-0,17	0,07	-2,45	645	0,04352197
P47709	Rabphilin-3A	RP3A	-0,16	0,07	-2,49	183	0,04127707
P06761	78 kDa glucose-regulated protein	GRP78	0,15	0,04	3,48	435	0,00280816
Q9QXQ0	Alpha-actinin-4	ACTN4	0,16	0,06	2,94	374	0,01364871
P24368	Peptidyl-prolyl cis-trans isomerase B	PIIB	0,30	0,10	3,08	99	0,01100049
P31044	Phosphatidylethanolamine-binding protein 1	RKIP	0,30	0,11	2,72	228	0,02394697
Q63081	Protein disulfide-isomerase A6	PDI6A	0,31	0,10	3,13	152	0,00878527
Q5X1E0	Acidic leucine-rich nuclear phosphoprotein 32 family member E	AN32E	0,33	0,09	3,73	6	0,03126756
P52555	Endoplasmic reticulum resident protein 29	ERP29	0,34	0,05	6,36	66	2,72E-07
P07153	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1	RPN1	0,35	0,07	5,83	88	1,06E-06
P04785	Protein disulfide-isomerase	PDI1A	0,40	0,09	4,39	296	0,00012347
Q8CGU6	Nicastrin	NICA	0,44	0,15	2,96	13	0,03523719
P37361	Methionine-3	MT3	0,48	0,16	2,93	20	0,02744153
Q4AEF8	Coatomer subunit gamma-1	COPG1	0,52	0,13	3,86	41	0,00216109
Q6RUV5	Ras-related C3 botulinum toxin substrate 1	RAC1	0,53	0,17	3,22	67	0,00838511
P14562	Lysosome-associated membrane glycoprotein 1	LAMP1	0,61	0,11	5,37	45	2,41E-05
Q91ZN1	Coronin-1A	COR1A	0,63	0,10	6,53	92	4,78E-08
P00787	Cathepsin B	CATB	0,74	0,16	4,60	185	6,78E-05
A8WCF8	Tumor protein p63-regulated gene 1-like protein	TPRGL	0,87	0,30	2,89	37	0,02253646
Q6GHD0	Endoplasmic reticulum protein	ENRPL	1,20	0,17	7,17	74	6,82E-09
P17475	Alpha-1-antitrypsin	A1AT	1,57	0,07	22,55	109	0
P38659	Protein disulfide-isomerase A4	PDI4A	1,69	0,09	18,91	52	0
Q6AYC4	Macrophage-capping protein	CAPG	1,74	0,10	17,12	101	0
P49134	Integrin beta-1	ITB1	4,55	0,12	37,16	26	0
P06238	Alpha-2-macroglobulin	A2MG	8,08	0,04	188,46	13	0
Q9ESR9	ATP-binding cassette sub-family A member 2	ABCA2	9,76	0,02	406,61	7	0
GO:0060627 regulation of vesicle-mediated transport							
ID	Protein	Short Name	Fold	Std Error	t value	DF	p value
Q9JIR3	Regulating synaptic membrane exocytosis protein 3	RIMS3	-1,046	0,08488	-12,33	5	0,0004234
Q9CWN8	Spectrin beta chain, brain 2	SPTN2	-0,696	0,10844	-6,419	92	7,73E-08
P08082	Clathrin light chain B	CLCB	-0,464	0,06083	-7,628	16	9,84E-06
P08082	Clathrin light chain B	CLCB	-0,464	0,06083	-7,628	16	9,84E-06
Q9IKE3	Secretory carrier-associated membrane protein 5	SCAM5	-0,454	0,09517	-4,767	16	0,00126997
P62824	Ras-related protein Rab-3C	RAB3C	-0,45	0,12808	-3,512	53	0,00443394
P10824	Guanine nucleotide-binding protein (G(i)) subunit alpha-1	GNAI1	-0,387	0,05606	-6,907	38	4,02E-07
O35964	Endophilin-A2	SH3G1	-0,363	0,09542	-3,804	56	0,00194406
P29101	Synaptotagmin-2	SYT2	-0,363	0,09932	-3,654	237	0,00179161
P61265	Syntaxin-1B	STX1B	-0,354	0,09272	-3,816	336	0,00100266
Q9Z5N3	PEX5-related protein	PEX5R	-0,347	0,10823	-3,203	14	0,0225164
P21707	Synaptotagmin-1	SYT1	-0,33	0,07792	-4,241	120	0,00030561
Q6RUV5	Reticulon-3	RTN3	-0,319	0,05911	-5,398	144	2,88E-06
P63012	Ras-related protein Rab-3A	RAB3A	-0,289	0,0949	-3,049	142	0,01117429
P05712	Ras-related protein Rab-2A	RAB2A	-0,272	0,06084	-4,479	190	0,00010494
P61023	Calcium-binding protein p22	CHP1	-0,263	0,0995	-2,648	45	0,03523719
P11442	Clathrin heavy chain 1	CLH	-0,254	0,03929	-6,464	1787	2,10E-09
Q99N27	Sorting nexin-1	SNX1	0,146	0,05728	2,5422	88	0,03961777
Q62940	E3 ubiquitin-protein ligase NEDD4	NEDD4	0,204	0,0639	3,1972	83	0,00838511
P17077	60S ribosomal protein L9	RL9	0,419	0,13978	2,9957	81	0,01390317
P02650	Apolipoprotein E	APOE	0,438	0,08778	4,9893	183	1,32E-05
P63025	Vesicle-associated membrane protein 3	VAMP3	0,467	0,0646	7,2357	7	0,0010564
Q4AEF8	Coatomer subunit gamma-1	COPG1	0,516	0,1338	3,8565	41	0,00216109
Q6RUV5	Ras-related C3 botulinum toxin substrate 1	RAC1	0,535	0,16598	3,231	67	0,00838511
P61751	ADP-ribosylation factor 4	ARF4	0,558	0,11304	4,9402	9	0,00397693
Q91ZN1	Coronin-1A	COR1A	0,626	0,09578	6,5329	92	4,78E-08
Q66H80	Coatomer subunit delta	COPD	0,831	0,17141	4,8493	69	6,36E-05

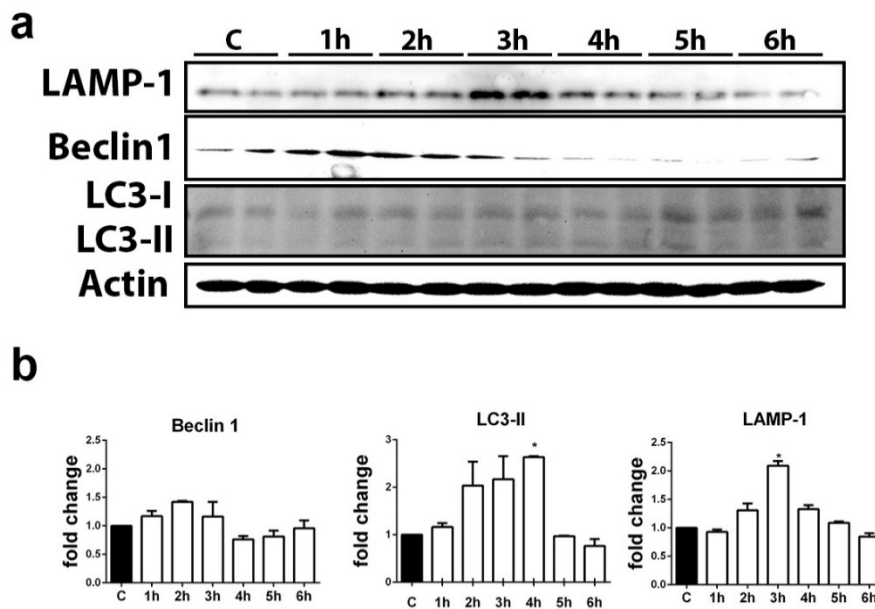
SUPPLEMENTARY FIGURES AND LEGENDS

Supplementary Figure S1



Supplementary Fig. S1. Western blot with anti-Lamp1 using sample of L4-L5 spinal cord extract from a control rat (C) and the same after being deglycosylated for 18h (C_{deglyc}). Note totally shift of band pattern.

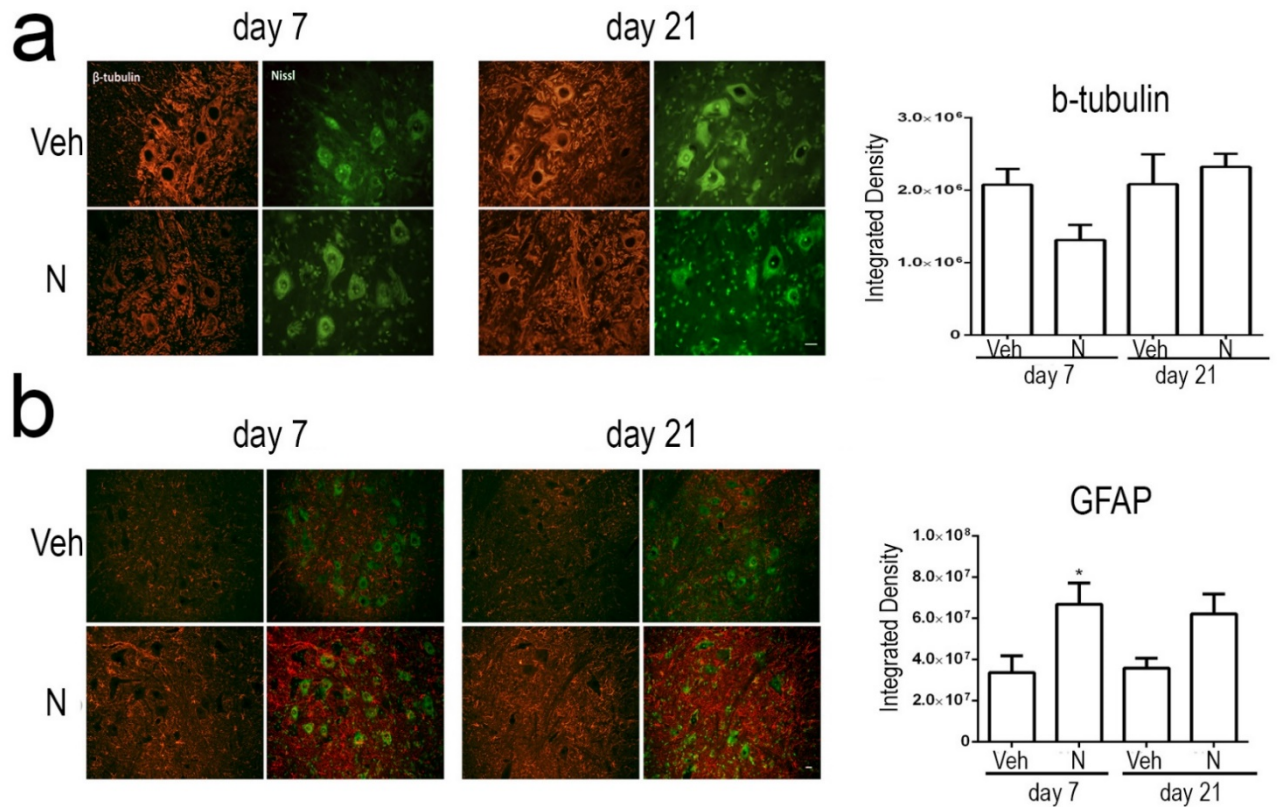
Supplementary Figure S2



Supplemental Figure S2. Ncs34 cell time-course response to rapamycin treatment. (a) Immunoblotting of key autophagy-related molecules after 10 μ M rapamycin added to the culture

medium for different time exposition. **(b)** Bar graph of fold change of Beclin, LC3II and LAMP1 at different time-points respect to actin values.

Supplementary Figure S3



Supplementary Figure S3. **(a) Left**, Representative microphotographs of β -tubulin (red) and green fluorescent Nissl of MNs at the ventral horns of the spinal cord from animals receiving either vehicle (Veh) or nocodazole (N) injection at 7 or 21 days post injection. **Right**, Bar graphs of the integrated density for β -tubulin within MNs at the different conditions. **(b)** Images and bar graphs of immunoreactivity analysis for GFAP staining (red) at the ventral horns with MNs labelled in green (Nissl) at the same conditions described. Scale bar= 25 μ m.