Supplementary Information for
Chemical and Genetic Rescue of In Vivo Progranulin-Deficient Lysosomal and Autophagic Defects

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Compound name	Effective in *C. elegans*	Effective in human NSC34 cells	Known functions	BBB permeability	References
Rivastigmine	✓	✓	Acetylcholinesterase inhibitor	✓	Int J Mol Sci. 2014 Jun; 15(6): 9809–9825
Rottlerin	✓	✓	Protein kinase C inhibitor	Unknown	Neurosci Lett. 2010 Jan; 468(3): 254-8
Resveratrol	✓	×	SIRT1 activator	✓	Front Aging Neurosci. 2014 Sep; 6: 218
Pirenzepine dihydrochloride	✓	×	Acetylcholine receptor agonist	✓	Scand J Gastroenterol Suppl. 1980; 66: 35-7
Azatadine maleate	✓	×	Histamine H1 receptor antagonist	✓	Contemporary Drug Synthesis. 2004
(±)-gamma-Vinyl GABA	✓	×	GABA transamniase inhibitor	✓	Br J Clin Pharmacol. 2014 Nov; 78(5): 981–995
XCT790	✓	×	Estrogen-related receptor alpha inverse agor	Unknown	Biochemistry. 2014 Jul; 53(29): 4839-46
Ethopropazine hydrochloride	✓	×	Anti-parkinsonian	Unknown	J Nerv Ment Dis. 1948 Aug; 108(2): 118-28
Methantheline bromide	✓	×	Antispastic	✓	Ann Pharmacother. 1995 May; 29(5): 489-92
Daurisoline	✓	×	Anti-inflammatory, hERG channel blocker	✓	Neuroreport. 1994 Jul; 5(12): 1489-92
PAPP	✓	×	Serotonin receptor agonist	Unknown	Advances in Drug Research (Vol. 17), 2013
Bay 11-7085	✓	×	Inhibitor of NFkB	Unknown	Exp Hematol. 2007 Oct; 35(10): 1495-509
(-)-Eseroline fumarate salt	×		Acetylcholinesterase inhibitor	×	Enzyme Inhib Med Chem. 2002 Oct; 17(5): 279-85
Clonixin Lysinate	×		Anti-inflammatory	Unknown	Clin Pharmacol New Drugs. 1971 Sep; 11(5): 371-7
Methylhydantoin-5-(D)	×		Anti-epileptic	Unknown	J Nerv Ment Dis. 1948 Aug; 108(2): 118-28
Tomatidine	×		Functional acid sphingomyelinase inhibitor	Suggested	PLoS One. 2011; 6(8): e23852
Verruculogen	×		Potassium channel blocker	✓	Mar Drugs. 2016 Nov; 14(11): 208
Oxyphenbutazone			Anti-inflammatory	✓	Eur J Clin Pharmacol. 1983; 25: 107
DL-alpha-Methyl-p-tyrosine			Tyrosine hydroxylase inhibitor	✓	Br J Pharmac. 1986; 88: 285-290
Dopamine hydrochloride			Various uses, primarily for low blood pressu	×	Drug Discov Today. 2007 Jan; 12(1-2): 54-61
Oxaprozin			Anti-inflammatory	Unknown	pert Opin Pharmacother. 2005 May; 6(5): 777-85
Tyrphostin 23			EGFR inhibitor	Unknown	Neurochem Res. 2016 Oct; 41(10): 2607-18
Fursultiamine Hydrochloride			Used for vitamin B1 deficiency	Unknown	Med Sci Monit. 2004 Sep; 10(9):199-203
Imatinib			Tyrosine kinase inhibitor	Poor	Blood. 2008 Aug 15; 112(4): 1005-12
Candesartan			Angiotensin II receptor antagonist	✓	Curr Med Res Opin. 2003; 19(5): 449-51
Idazoxan hydrochloride			Adrenergic receptor antagonist	✓	Anesthesiology. 1989 Jul; 71(1): 75-9
Daphnetin			Protein kinase inhibitor	Unknown	Eur J Pharmacol. 2011 Oct; 668(1-2): 35-41
Viloxazine hydrochloride			Norepinephrine reuptake inhibitor	✓	Analogue-based Drug Discovery II, 2010
Lamotrigine			Voltage-gated sodium channel inhibitor	✓	Biochem Pharmacol. 2012 Mar; 83(6): 805-14
(S)-3,5-Dihydroxyphenylglycine			Glutamate receptor agonist	Unknown	CNS Drug Rev. 2002; 8(1): 101-16
Carmustine			DNA alkylating agent	✓	Handbook of Brain Tumor Chemotherapy, 2006
Promethazine hydrochloride			Histamine H1 receptor antagonist	✓	Arch Dermatol Res. 2012 May; 304(4): 263–272
Pizotifen malate			Serotonin receptor antagonist	✓	Science and Practice in Clinical Neurology, 1993
Zuclopenthixol dihydrochloride			Dopamine receptor antagonist	✓	Drugs in Psychiatry, 2013
Meropenem	Cannot test in *C. elegans*		Antibacterial	✓	Clin Microbiol Rev. 2010 Oct; 23(4): 858-883

Table S1. Compounds identified from *C. elegans* drug screen and their properties

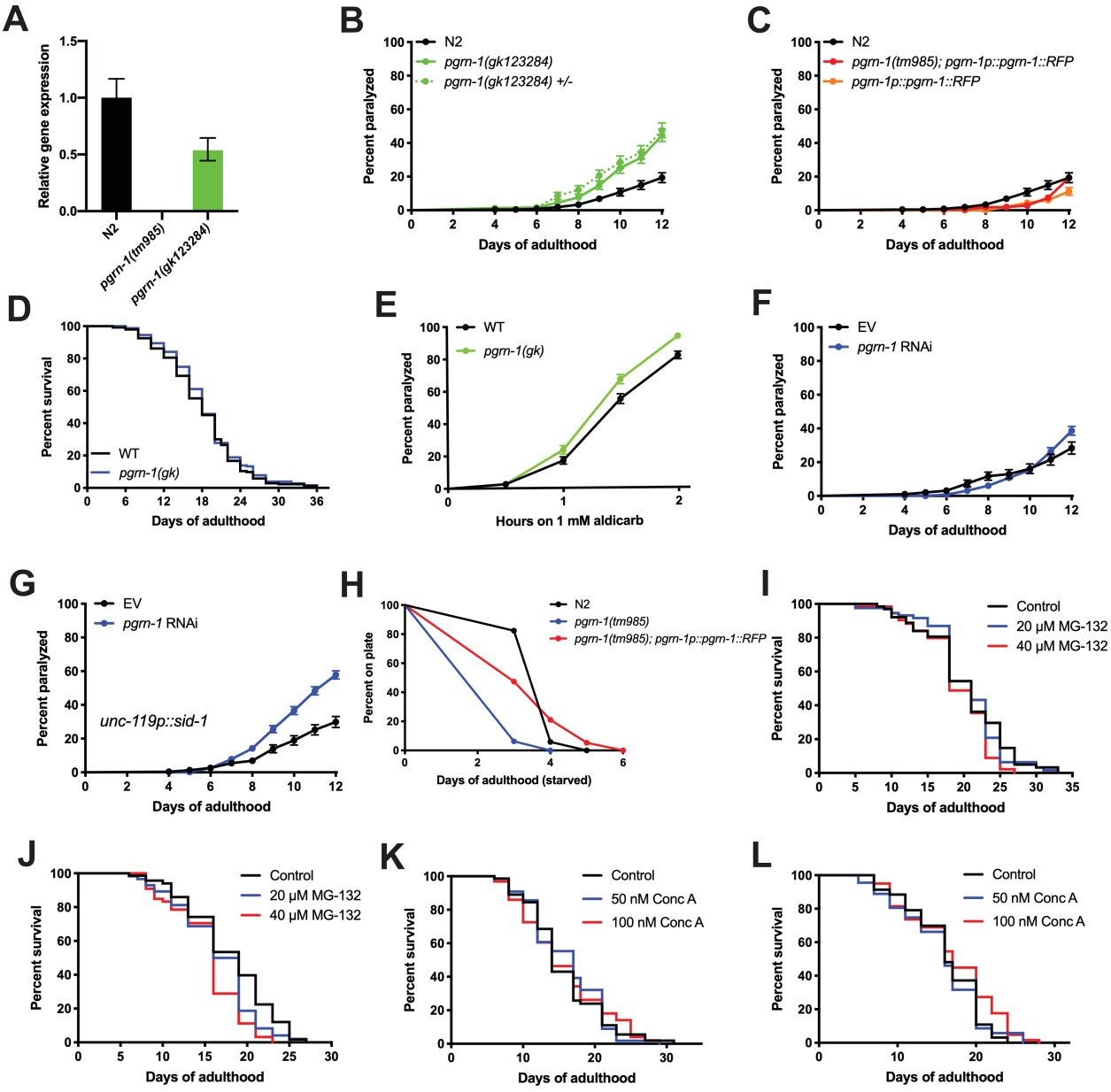

Figure S1. (A) Quantitative gene expression reveals that *pgrn-1(gk)* animals show a marked reduction in *pgrn-1* mRNA expression, while it could not be detected in *pgrn-1(tm985)* mutants (Student's *t* test: N2 vs. *pgrn-1(tm985)*, ****P<0.0001; N2 vs. *pgrn-1(gk)*, ****P<0.0001). (B) *pgrn-1(gk)* animals are display higher levels of age-dependent paralysis than N2 animals, and heterozygous *pgrn-1(gk)*/+ animals display the same levels of paralysis as homozygous mutant

animals (Mantel-Cox test, N2 vs. *pgrn-1(gk)*, ****P<0.0001; *pgrn-1(gk)* vs. *pgrn-1(gk)*/+, n.s.). (C) Overexpression of *pgrn-1::rfp* in a *pgrn-1(tm985)* background display similar levels of paralysis as N2 animals, whereas expressed in a wild-type background results in a slight decrease in paralysis (Mantel-Cox test: N2 vs. *pgrn-1(tm985); pgrn-1::rfp*, n.s.; N2 vs. *pgrn-1::rfp*, *P<0.05). (D) Lifespan in unaffected in *pgrn-1(gk)* animals (Mantel-Cox test, n.s.). (E) *pgrn-1(gk)* animals display heightened aldicarb hypersensitivity compared to N2 animals (Mantel-Cox test, ****P<0.0001). (F-G) Knockdown of *pgrn-1* in N2 animals (F) does not result in an increase in paralysis, while the knockdown in neurons does (G) (Mantel-Cox test: RNAi in N2 (F), n.s.; neuronal RNAi (G), ****P<0.0001). (H) Nematodes lacking *pgrn-1* display an overactive food-seeking behavior and crawl off NGM plates faster than N2 controls; this phenotype is partially rescued by re-expression of *pgrn-1::rfp* (Mantel-Cox test: N2 vs. *pgrn-1(tm985)*, ****P<0.0001; *pgrn-1(tm985)* vs. *pgrn-1(tm985); pgrn-1::rfp*, **P<0.01). (I) Treatment of N2 animals with either 20 or 40 uM MG-132 did not result in a decrease in lifespan (Mantel-Cox test: Vehicle vs. 20 uM, n.s.; Vehicle vs. 40 uM, n.s.). (J) Treatment of *pgrn-1::rfp* animals with 20 uM MG-132 did not result in a decrease in lifespan but treatment with 40 uM MG-132 did (Mantel-Cox test: Vehicle vs. 20 uM, n.s.; Vehicle vs. 40 uM, ****P<0.001.). (K) Treatment of N2 animals with either 50 or 100 nM Concanamycin A did not result in a decrease in lifespan (Mantel-Cox test: Vehicle vs. 50 nM, n.s.; Vehicle vs. 100 nM, n.s.). (L) Treatment of *pgrn-1::rfp* animals with either 50 or 100 nM Concanamycin A did not result in a decrease in lifespan (Mantel-Cox test: Vehicle vs. 50 nM, n.s.; Vehicle vs. 100 nM, n.s.).

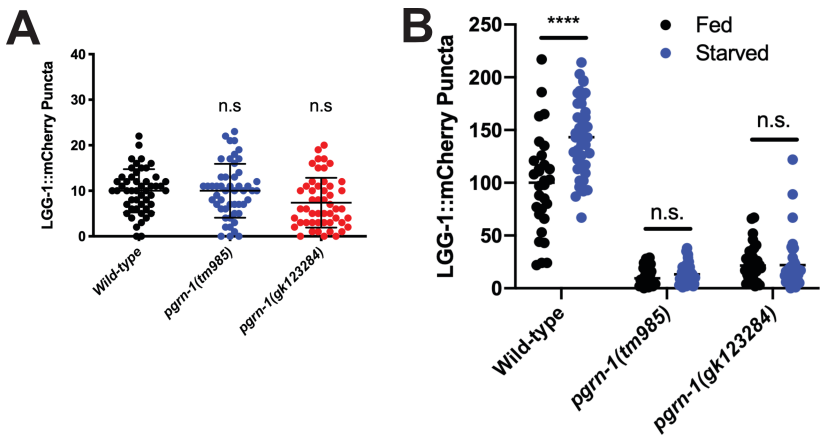

Figure S2. (A) Both *pgrn-1* mutations did not alter LGG-1::mCherry puncta formation at day 1 of adulthood (Student's *t* test: WT vs. *pgrn-1(tm985)*, n.s.; WT vs. *pgrn-1(gk123284)*, n.s.). (B) Starvation conditions induced autophagy in WT animals, but had no effect on autophagy in both *pgrn-1* mutants (Student's *t* test, Fed vs. Starved: WT, ****$p<0.0001$; *pgrn-1(tm)*, n.s.; *pgrn-1*(gk), n.s.).

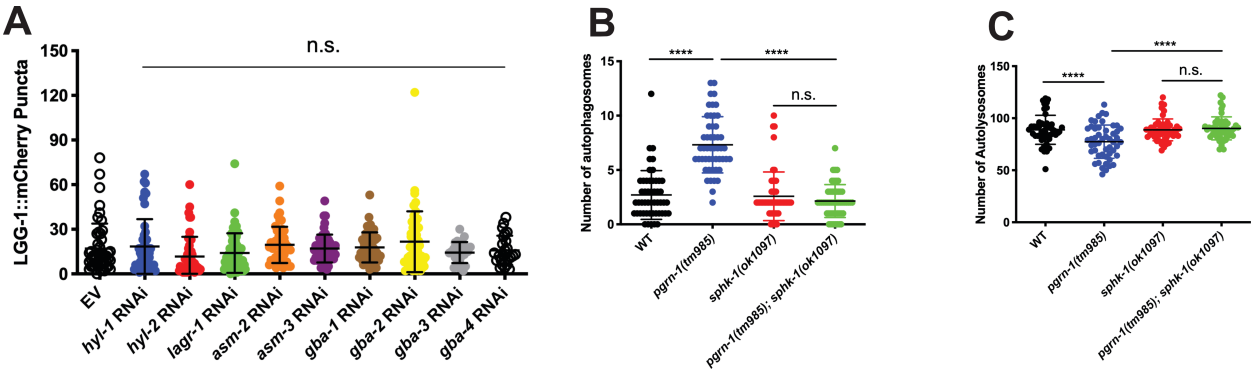

Figure S3. (A) RNAi knockdown of the corresponding genes did not significantly affect formation of LGG-1::mCherry puncta in *pgrn-1(tm985)* mutants. (B-C) The genetic double mutant, *sphk-1(ok1097); pgrn-1(tm)*, had restored levels of autophagosomes (B) and autolysosomes (C) in animals' neurons (One-way ANOVA, autophagosomes: WT vs. double mutant, n.s.; double mutant vs. *pgrn-1(tm)*, ****p<0.0001; autolysosomes: WT vs. double mutant, n.s.; double mutant vs. *pgrn-1(tm)*, ****p<0.0001).

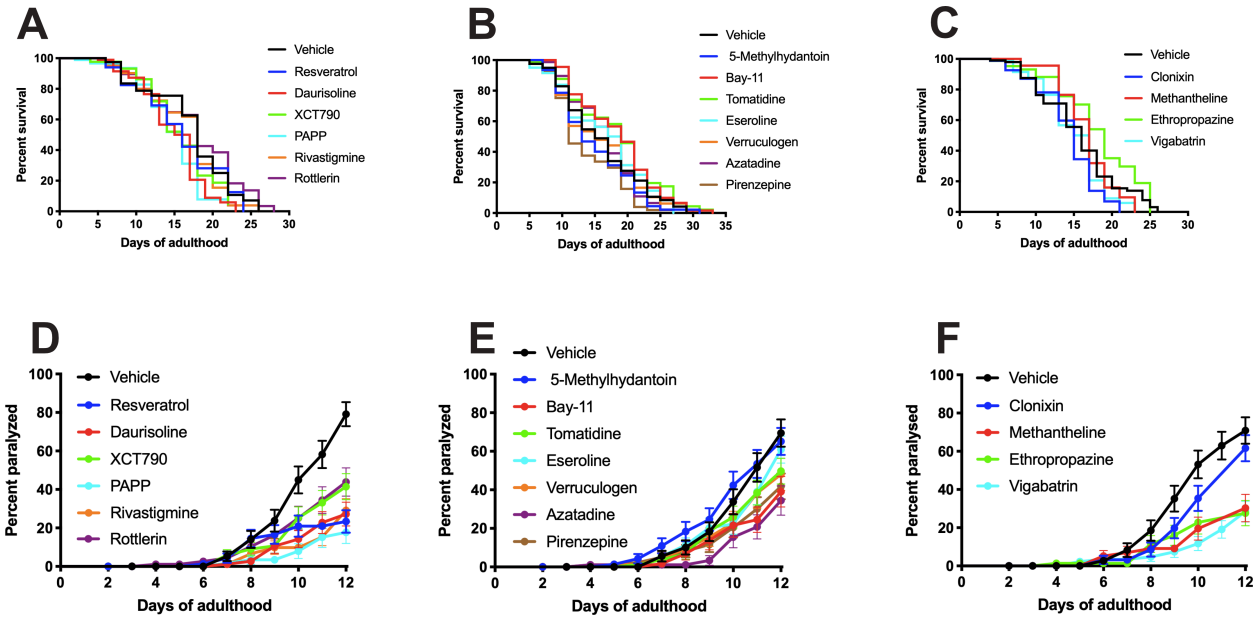

Figure S4. (A-C) Validation of top 17 drugs from the liquid culture screen for their ability to influence lifespan in *pgrn-1(tm)* mutants (Mantel-Cox test, treatment condition vs. Vehicle control. A: Resveratrol, n.s.; Daurisoline, $^{**}p<0.01$; XCT790, n.s.; PAPP, n.s.; Rivastigmine, n.s.; Rottlerin, n.s. B: 5-Methylhydandoin, n.s.; Bay-11, n.s.; Tomatidine, n.s.; Eseroline, n.s.; Verruculogen, n.s.; Azatadine, n.s.; Pirenzepine, $^{*}p<0.05$. C: Clonixin, n.s.; Methantheline, $^{**}p<0.01$; Ethropropazine, $^{****}p<0.0001$; Vigabatrin, n.s.). (D-F) Validation of top 17 drugs on the paralysis phenotype exhibited by *pgrn-1(tm)* nematodes (Mantel-Cox test, treatment condition vs. Vehicle control. A: Resveratrol, $^{****}p<0.0001$; Daurisoline, $^{****}p<0.0001$; XCT790, $^{***}p<0.001$; PAPP, $^{****}p<0.0001$; Rivastigmine, $^{****}p<0.0001$; Rottlerin, $^{**}p<0.01$. B: 5-Methylhydandoin, n.s.; Bay-11, $^{*}p<0.05$; Tomatidine, n.s.; Eseroline, n.s.; Verruculogen, n.s.; Azatadine, $^{***}p<0.001$; Pirenzepine, $^{*}p<0.05$. C: Clonixin, n.s.; Methantheline, $^{***}p<0.001$; Ethropropazine, $^{***}p<0.001$; Vigabatrin, $^{****}p<0.0001$).

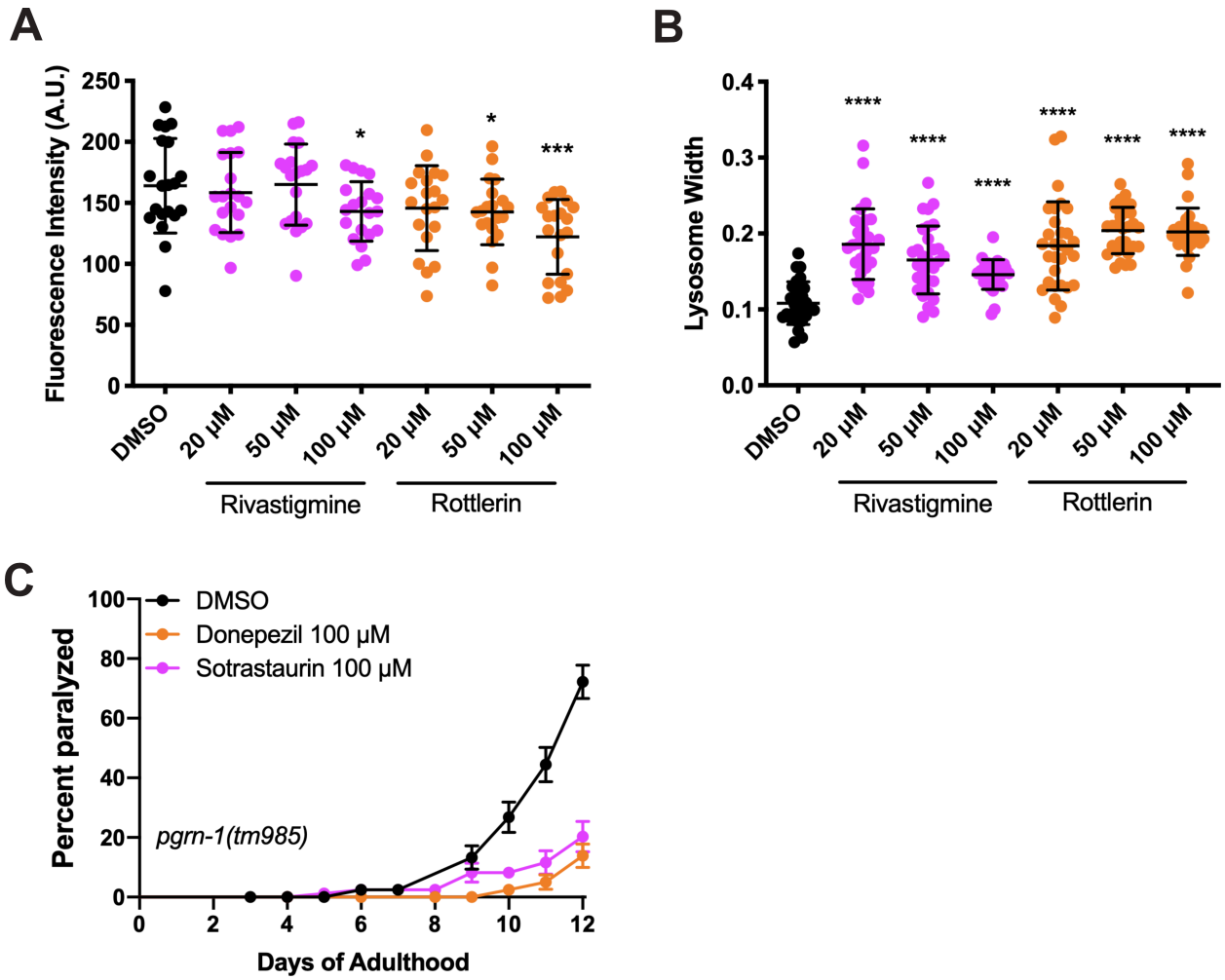

Figure S5. (A-B) Dose-dependent testing of rottlerin and rivastigmine against lysosomal phenotypes (Student's *t* test, A: DMSO control vs. treatment condition: 100 μM rivastigmine *p<0.05, 50 μM rottlerin *p<0.05, 100 μM Rottlerin ***p<0.001; B: DMSO control vs. all treatment conditions, ****p<0.0001). (C) Donepezil and sotrastaurin restore paralysis phenotypes in *pgrn-1(tm)* animals (Mantel Cox test, ****p<0.0001)