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### **Citation**

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**Note:** To cite this publication please use the final published version (if applicable).

## RESEARCH ARTICLE

# Progranulin deficiency leads to reduced glucocerebrosidase activity

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## OPEN ACCESS

**Citation:** Zhou X, Paushter DH, Pagan MD, Kim D, Nunez Santos M, Lieberman RL, et al. (2019) Progranulin deficiency leads to reduced glucocerebrosidase activity. *PLoS ONE* 14(7): e0212382. <https://doi.org/10.1371/journal.pone.0212382>

**Editor:** Thomas Kukar, Emory University School of Medicine, UNITED STATES

**Received:** January 31, 2019

**Accepted:** June 19, 2019

**Published:** July 10, 2019

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**Data Availability Statement:** All relevant data are within the manuscript and its Supporting Information files.

**Funding:** This work is supported by funding to F. Hu from National Institute of Aging and National Institute of Neurological Disorders and Stroke (R01 NS095954, NS088448) and President's Council of Cornell Women Affinity-Stewart award.

**Competing interests:** The authors have declared that no competing interests exist.

## Abstract

Mutation in the *GRN* gene, encoding the progranulin (PGRN) protein, shows a dose-dependent disease correlation, wherein haploinsufficiency results in frontotemporal lobar degeneration (FTLD) and complete loss results in neuronal ceroid lipofuscinosis (NCL). Although the exact function of PGRN is unknown, it has been increasingly implicated in lysosomal physiology. Here we report that PGRN interacts with the lysosomal enzyme, glucocerebrosidase (GCase), and is essential for proper GCase activity. GCase activity is significantly reduced in tissue lysates from PGRN-deficient mice. This is further evidence that reduced lysosomal hydrolase activity may be a pathological mechanism in cases of *GRN*-related FTLD and NCL.

## Introduction

Progranulin (PGRN), encoded by the *GRN* gene in humans, is a glycoprotein comprised of 7.5 conserved and highly disulfide-bonded homologous granulin domains connected by short linker regions [1–6]. While the exact function of PGRN remains elusive, it has been found to be involved in numerous normal physiologic and pathologic processes, including regulation of inflammation, wound healing, and tumorigenesis, and it has also been shown to function as a growth and neurotrophic factor [7–16]. Mutation in *GRN* has been linked to two neurodegenerative diseases with dose-dependent associations. Heterozygous mutation, resulting in PGRN haploinsufficiency, is known to cause frontotemporal lobar degeneration (FTLD), a clinically and pathologically heterogeneous disease often resulting in early-onset dementia [17–23]. Homozygous mutation, resulting in complete loss of PGRN, causes neuronal ceroid lipofuscinosis (NCL), a lysosomal storage disease [24, 25]. Although these two diseases are distinct, NCL-related phenotypes have been reported in FTLD patients with *GRN* mutation, suggesting that lysosomal dysfunction might serve as a common mechanism [26–28].

**Abbreviations:** FTLD, frontotemporal lobar degeneration; NCL, neuronal ceroid lipofuscinosis; PGRN, progranulin; GCCase, glucocerebrosidase; PSAP, prosaposin; SAP, saposin; SILAC, stable isotope labeling by amino acids in cell culture.

In addition to the connection to NCL, increasing lines of evidence indicate that PGRN is likely to be involved in lysosomal physiology. In addition to being lysosomally localized, having two independent trafficking pathways to the lysosome [29, 30], and being under transcriptional regulation with the majority of known lysosomal proteins [31], PGRN has recently been shown to be proteolytically processed to produce stable and functional granulin peptides in the lysosome [32–34], and is linked to the direct or indirect regulation of two lysosomal hydrolases, cathepsin D (CTSD) and glucocerebrosidase (GCCase, encoded by the *GBA* gene). PGRN was shown to stabilize and directly modulate the activity of cathepsin D *in vitro* and PGRN deficiency results in a reduction of cathepsin D activity *in vivo* [28, 35, 36]. In another study, PGRN was purported to be a co-chaperone of glucocerebrosidase (GCCase) [37, 38], a  $\beta$ -glucosidase mutated in Gaucher disease, in tandem with heat shock protein 70 (HSP70) [39]. *Grn* knockout mice with induced chronic inflammation display cellular phenotypes similar to those with Gaucher disease [37, 38].

To better understand the lysosomal role of PGRN, we performed a stable isotope labeling by amino acids in cell culture (SILAC)-based proteomic screen for PGRN protein interactors. Corresponding with the previous findings, one of the top hits was GCCase. In this study, we demonstrate that PGRN loss results in a substantial decrease in GCCase activity in mouse tissues without any changes in protein levels.

## Material and methods

### Primary antibodies and reagents

The following antibodies were used in this study: M2 mouse anti-FLAG (Sigma), M2 mouse anti-FLAG-conjugated beads (Sigma), mouse anti-myc (9E10), mouse anti-GAPDH (Protein-tech Group), mouse anti-GBA (MilliporeSigma), rat anti-mouse LAMP1 (BD Biosciences), rabbit anti-calnexin (Abcam) and sheep anti-mouse PGRN (R&D Systems). Rabbit anti-mouse PSAP and PGRN antibodies were produced as previously described [30]. GFP-Trap and Myc-Trap beads were from ChromoTek. 4-Methylumbelliferyl  $\beta$ -D-glucopyranoside (4-MU), GCCase substrate, were obtained from Sigma.

### Plasmids

Human GBA cDNA in the pDONR223 vector from the human ORFeome 8.1 collection was a gift from Dr. Haiyuan Yu (Cornell University). GBA was cloned into pcDNA3.1/myc-His A vector (Thermo Fisher Scientific) after digestion with EcoRI and XhoI. Human PGRN in the pCMV-Sport6 vector was obtained as previously described [29]. GFP-PGRN was produced as previously described [30]. GFP-granulin peptides were produced as described [36]. Mouse GBA with C-terminal FLAG myc tag was obtained from Origene.

### Protein production and purification

GST-granulin E, GST-granulin F and His-SUMO-saposin C proteins were expressed from Origami B(DE3) bacterial strains (MilliporeSigma) with 0.1 mM IPTG induction overnight at 18°C. Proteins were purified using GST or cobalt beads. His-PGRN was purified with cobalt beads from the culture media of HEK293T cells as previously described [30]. All purified proteins were concentrated and transitioned to PBS buffer with Centricon Centrifugal Filter Units (MilliporeSigma).

### Mouse strains

C57/BL6 and *Grn*<sup>-/-</sup> mice [40] were obtained from The Jackson Laboratory. *Gba*<sup>-/-</sup> mice were generated as previously described [41]. Mixed male and female mice were used for this study.

## Cell culture

HEK293T and BV2 cells were maintained in Dulbecco's Modified Eagle's Medium (Cellgro) supplemented with 10% fetal bovine serum (Gibco) in a humidified incubator at 37°C with 5% CO<sub>2</sub>. WT, *Grn*<sup>-/-</sup> and *Gba*<sup>-/-</sup> mouse fibroblasts were cultured as described [30].

## Transfection, immunoprecipitation, and western blot analysis

Cells were transfected with polyethylenimine as previously described [42]. Cells were lysed in a cold, near-neutral pH solution containing 150 mM NaCl, 50 mM Tris pH 7.5 or 50mM sodium acetate pH5.3, 1% Triton X-100, 0.1% deoxycholic acid, 1X protease inhibitors (Roche). After centrifugation at 14,000 xg, for 15 minutes, at 4°C, supernatants were transferred to clean tubes on ice, to which rabbit anti-PGRN antibody-conjugated Affi-Gel 15 (Bio-Rad Laboratories), Myc-Trap or GFP-Trap beads were added, then rocked for 3–4 hours at 4°C. Samples were run on 12% polyacrylamide gels or 4–12% Bis-Tris gels (Invitrogen), then transferred to Immobilon-FL polyvinylidene fluoride membranes (Millipore Corporation) or nitrocellulose membranes (Millipore Corporation). Membranes were blocked with either 5% non-fat milk in PBS or Odyssey Blocking Buffer (LI-COR Biosciences) for 1 hour then washed with Tris-buffered saline with 0.1% Tween-20 (TBST) 3x for 5 minutes each. Membranes were incubated with primary antibodies, rocking overnight at 4°C, then washed as above, incubated with secondary antibodies for 2 hours at room temperature, then washed again. Membranes were scanned using an Odyssey Infrared Imaging System (LI-COR Biosciences). Densitometry was performed with Image Studio (LI-COR Biosciences).

## Tissue preparation for enzyme assays and western blot

2-month- old WT and *Grn*<sup>-/-</sup> mice were perfused and tissues were dissected and snap-frozen with liquid nitrogen and kept at -80C. On the day of the experiment, frozen tissues were thawed and homogenized on ice with a glass Dounce homogenizer in a cold solution of either 1% (w/v) sodium taurocholate and 1% (v/v) Triton X-100, pH 5.2, for 4-MU activity assays or 0.2% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 for MDW941 assays. Protein concentrations were determined via Bradford assay, then standardized.

## Lysosome isolation

Lysosomes were isolated from liver tissues of 2-month- old WT and *Grn*<sup>-/-</sup> mice with lysosome isolation kit according to the instructions with the following optiprep gradients ((8%, 12%, 16%, 19%, 23%, 27%). Lysosomes are enriched in fraction #2 (12%-16%).

## GCase activity assay with 4-MU substrate

Tissue lysates, prepared as described, were diluted in a cold buffer of 0.1 M citric acid/0.2 M disodium phosphate (pH 5.0), with 2 mg/mL bovine serum albumin added. Ten microliters of each sample were added to 75 µL of cold 10 mM 4-MU substrate in the same buffer and incubated at 37°C for 30 minutes. Reactions were stopped by the addition of 200 µL of a 0.3 M glycine/0.2 M sodium carbonate (pH 10.7) stop solution. Plates were read at 360 nm excitation/460 nm emission with an Infinite M1000 microplate reader (Tecan). To test for the direct activation of recombinant GCase, a 50 µL reaction mixture containing 1 mg/mL BSA, 0.1 M sodium acetate (pH 4.5), 0.02 U Cerezyme, 0.2 mM 4-MU substrate, 0.1% Triton X-100, and 1, 5, or 9 µM recombinant PGRN, Grn E, Grn F, saposin C, GST, or an equal volume of PBS was incubated at 37°C for 30 minutes. Reactions were stopped by the addition of 50µL of a 0.32 M

glycine/0.32 M sodium carbonate (pH 10.4) stop solution. Plates were read at 340 nm excitation/420 nm emission with an Infinite M1000 microplate reader (Tecan).

### Active GCase assessment with MDW941 probe

MDW941 was diluted to 100nM in tissue lysates, which were then incubated at 37°C for 30 minutes. Reactions were stopped by the addition of an equal volume of 2x Laemmli sample buffer with 10% β-Mercaptoethanol before heating at 95°C for 5 minutes. An equal amount of each sample (50μg total protein) was run on a 12% polyacrylamide gel, which was scanned at 532 nm excitation/580 nm emission with a Typhoon Imaging System (GE Healthcare), then western blot and assessment were performed as described above, with all values normalized to GAPDH.

### MDW941 cell labeling, immunostaining, and confocal microscopy

WT and *Grn*<sup>-/-</sup> MEFs were cultured on glass coverslips overnight. The next day, MDW941 was diluted to 5 nM in culture media, then equal volumes were added to each well and the plate was incubated at 37°C for 2 hours. Cells were washed 2x with PBS, fixed with 3.7% paraformaldehyde for 15 minutes at room temperature, followed by 3 additional PBS washes. Cells were permeabilized with Odyssey Blocking Buffer LI-COR Biosciences) + 0.05% saponin for 30 minutes at room temperature. Primary antibodies were diluted in the same buffer and added to coverslips, which were incubated in a humidified chamber overnight at 4°C. Coverslips were washed 3x with PBS, for 5 minutes each, then secondary antibodies diluted in the same blocking/permeabilization solution were added to the coverslips, which were incubated at room temperature, in the dark, for 2 hours. After 3 additional PBS washes, coverslips were mounted on slides with Fluoromount-G (SouthernBiotech). Images were acquired with a CSU-X series spinning disc confocal microscope (Intelligent Imaging Innovations) with an HQ2 CCD camera (Photometrics) using a 100x objective.

### SILAC and mass spectrometry

BV2 cells were infected with control lentivirus (pLenti-CRISPR2, Addgene) or lentivirus expressing Cas9 and guide RNA (sequence 5'-GCTCCCTGGGAGGCATCTGG-3') and selected with puromycin. The cells were then grown a minimum of five generations in DMEM with 10% dialyzed fetal bovine serum (Sigma) supplemented with either heavy (C13, N15 arginine and lysine) amino acids or light (C12, N14 arginine and lysine). Cells were grown to confluency in two 15-cm plates, each, and lysed then immunoprecipitated as described above using homemade rabbit anti-PGRN antibodies bound to Affi-Gel 15 (Bio-Rad Laboratories). Samples were then mixed and boiled 5 minutes with 10mM DTT followed by alkylation by treating samples with a final concentration of 28 mM iodoacetamide. Proteins were precipitated on ice for 30 minutes with a mixture of 50% acetone/49.9% ethanol/0.1% acetic acid. Protein was pelleted and washed with this buffer, reprecipitated on ice, and dissolved in 8 M urea/50 mM Tris (pH 8.0) followed by dilution with three volumes of 50 mM Tris (pH 8.0)/150 mM NaCl. Proteins were digested overnight at 37°C with 1 μg of mass spectrometry grade Trypsin (Promega). The resulting peptide samples were cleaned up for mass spectrometry in a Sep-Pak C18 column (Waters) as follow: samples were acidified with 0.25% formic acid and 0.25% trifluoroacetic acid, loaded onto a preequilibrated C18 column, washed twice with 0.1% acetic acid and eluted with 80% acetonitrile/0.1% acetic acid into silanized vials (National Scientific). Samples were evaporated using a SpeedVac and then redissolved in 70% acetonitrile with ~1% formic acid. Peptides were separated using hydrophilic interaction liquid chromatography on an Ultimate 300 LC (Dionex). Each fraction was evaporated in a SpeedVac and

resuspended in 0.1% trifluoroacetic acid with spiked-in angiotensin II as an internal standard. Samples were run on a QE Orbitrap XL mass spectrometer (Thermo Fisher Scientific). XPRESS software, part of the Trans-Proteomic Pipeline (Seattle Proteome Center), was used to quantify all the identified peptides. Mann-Whitney U test was used to calculate the p value to generate the volcano plot.

## Ethical approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The work under animal protocol 2017–0056 is approved by the Institutional Animal Care and Use Committee at Cornell University.

## Results

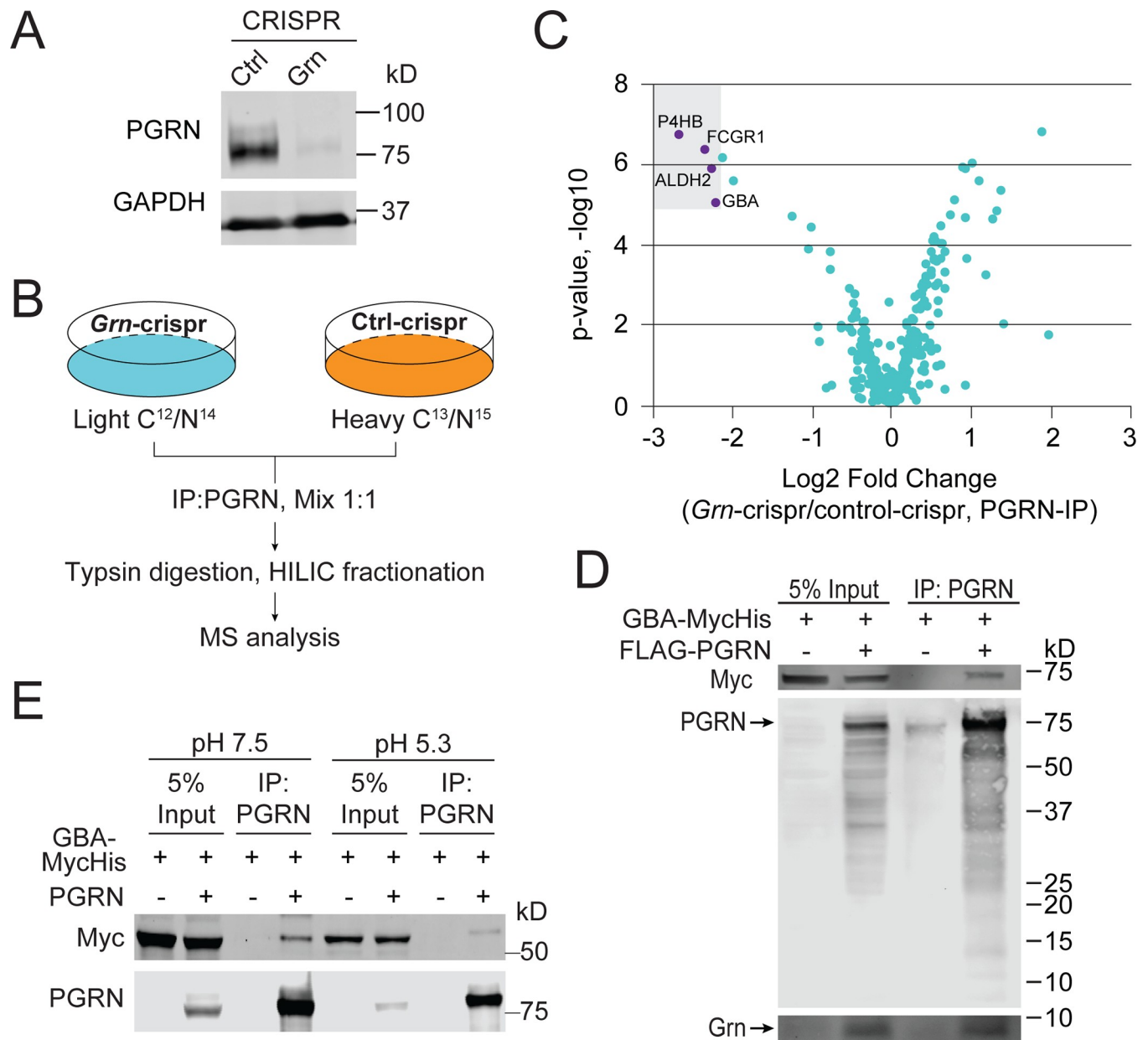
### PGRN interacts with GCase

SILAC was performed in the murine microglia-derived BV2 cell line, with either normal PGRN expression or loss of PGRN expression after CRISPR/cas9 mediated genome editing [43, 44] (Fig 1A, 1B and 1C). GCase (encoded by the *GBA* gene) was one of the high-confidence hits for PGRN from this experiment (Fig 1C) (S1 Table).

To verify the physical interaction between PGRN and GCase, FLAG-human PGRN and human GBA with a C-terminal myc tag were co-transfected in HEK293T cells, then anti-PGRN immunoprecipitation (IP) was performed. GBA-myc signal was detected in the IP products from the PGRN transfected, but not control samples, indicating a specific binding interaction (Fig 1D). This interaction persists at pH5.3 (Fig 1E). Similar interactions were detected with mouse PGRN and mouse GCase (S1 Fig). Additionally, PGRN has been shown to be processed into individual granulin peptides within the lysosome [32, 33] and two of these peptides, Grn E and Grn F, have been shown to interact with GCase [37, 38]. Since both PGRN and Grn peptides were pulled down in the anti-PGRN IP (Fig 1D), we tested whether GCase can bind specific Grn peptides. N-terminal GFP-tagged Grn peptides or GFP-PGRN were co-transfected with myc-tagged GBA, followed by anti-GFP IP. While there was no obvious binding of granulin E, granulin F and, to a lesser extent, granulin A did interact with GCase in the cell lysate (Fig 2A). However, it is not clear whether these granulin peptides are folded correctly when expressed individually. We repeated the experiments with secreted granulins, which, having proceeded through the entire secretory pathway, may be more likely to be properly folded. In this instance, all the granulins except Grn G shows weak binding to GCase (Fig 2B).

### PGRN deficiency leads to reduced GCase activities

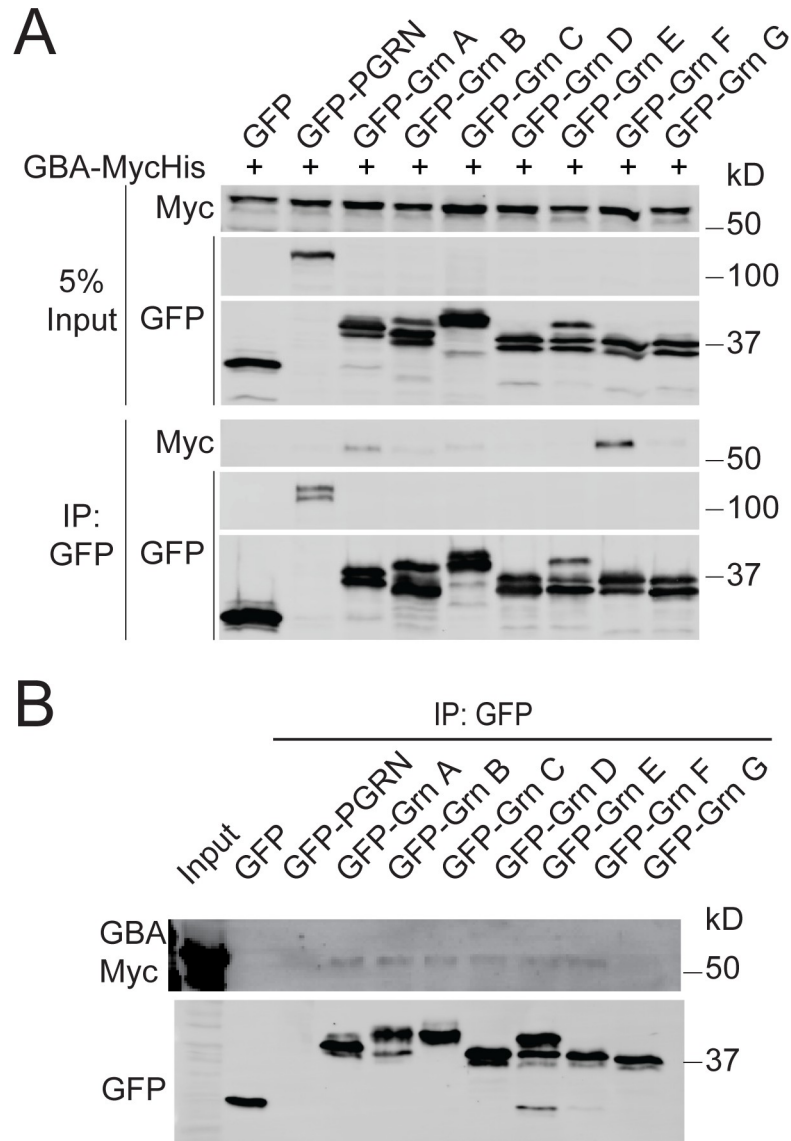
To determine whether PGRN regulates GCase activities, we performed an *in vitro* GCase activity assay using a well-established fluorogenic GCase substrate, 4-Methylumbelliferyl  $\beta$ -D-glucopyranoside (4MU- $\beta$ -glc) [45–47] (Fig 3). GCase activity was measured in tissue lysates from 2-month-old WT and *Grn*<sup>-/-</sup> mice, before obvious lysosomal phenotypes were observed. Liver and spleen lysates from *Grn*<sup>-/-</sup> mice showed a significant decrease in GCase activity compared to WT controls (Fig 3A and 3B). While the cerebrum and cerebellum showed a trend toward decreased GBA activity, it was not significant (Fig 3C and 3D). However, the midbrain, where we tend to observe the most severe defects in our knockout mouse line, showed a significant decrease in GCase activity (Fig 3E). Thus PGRN is likely to have cell type and tissue specific effects on GCase activities (S2 Table).



**Fig 1. PGRN interacts with GCase.** (A) Western blot analysis of PGRN levels in BV2 cells expressing control or guide RNA to the mouse *GRN* gene. (B) Schematic illustration of the SILAC experiment searching for PGRN interactors. (C) Volcano plot of SILAC hits. Top hits identified in the heavy fraction are highlighted. (D) Anti-PGRN co-immunoprecipitation of FLAG-PGRN and GBA-myc overexpressed in HEK293T cells. (E) Anti-PGRN co-immunoprecipitation of PGRN and GBA-myc overexpressed in HEK293T cells using buffers of pH 7.5 and pH 5.3.

<https://doi.org/10.1371/journal.pone.0212382.g001>

These results were confirmed using a recently developed ultrasensitive fluorescent probe, MDW941, which specifically reacts with active forms of the enzyme and has been shown to be sensitive enough to detect the activity of recombinant GCase in the attomolar range [48–50]. Using this probe, an even greater disparity between GBA activity in WT and *Grn*<sup>-/-</sup> tissues was observed (Fig 4A–4E, S2 Table). To verify that any changes in activity were not due to alterations in total GCase protein levels, SDS-PAGE and western blot of the tissue lysates were performed using antibodies specific to GCase (S2 Fig), with no significant differences seen between groups (Fig 4A–4E, S3 Fig, S2 Table).

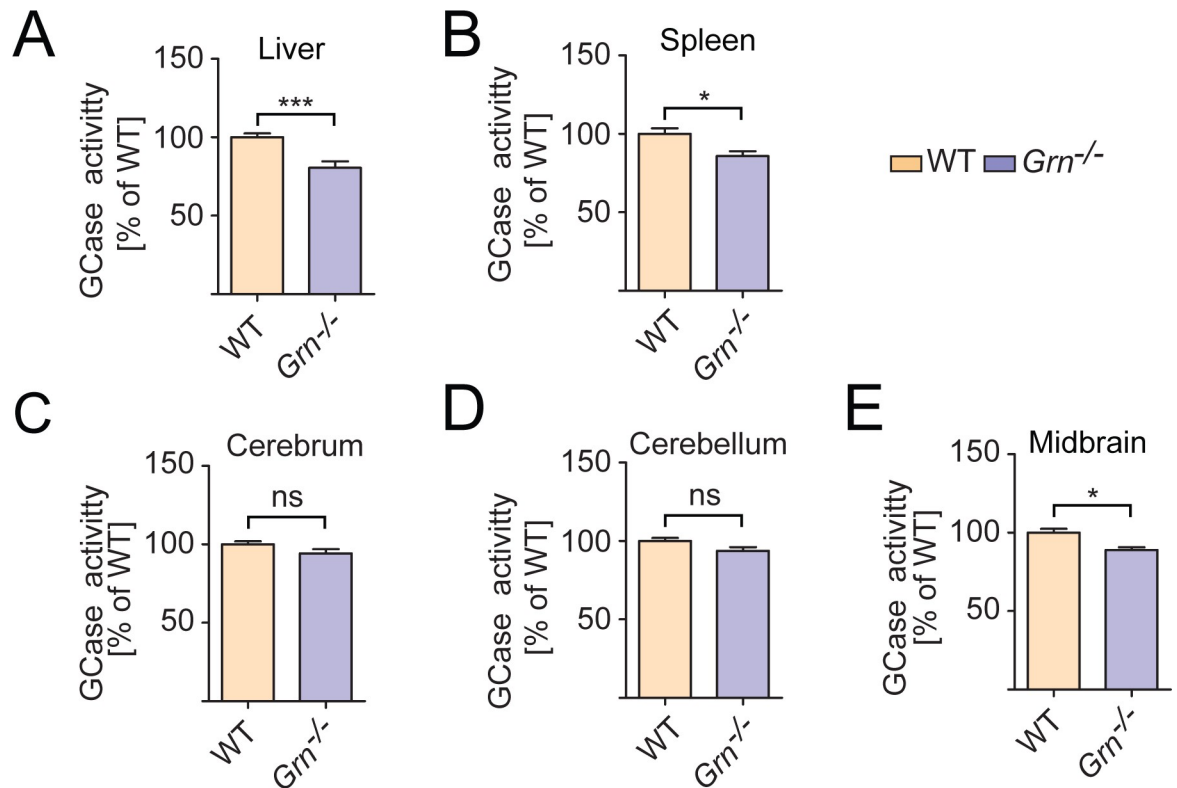


**Fig 2. Interaction between GCase and granulin peptides.** (A) Anti-GFP co-immunoprecipitation of GFP-PGRN or individual GFP-granulins and GBA-myc overexpressed in HEK293T cells shows binding primarily to Grn F, with weaker binding to Grn A and other Grn peptides. (B) Anti-GFP co-immunoprecipitation of GFP-PGRN or individual GFP-granulins and GBA-myc from conditioned medium of transfected cells shows weaker binding to all the granulins except Grn G.

<https://doi.org/10.1371/journal.pone.0212382.g002>

GCase was reported to aggregate in the cytosol in the absence of PGRN [37, 38]. To determine whether PGRN causes GCase trafficking defect, we performed lysosome isolation from WT and *Grn*<sup>-/-</sup> livers and blotted for GCase in the lysosome fraction (Fig 5), since none of commercially available antibodies for GCase gave specific signals in immunostaining. GCase is enriched in lysosome fractions in both WT and *Grn*<sup>-/-</sup> livers, suggesting that GCase lysosome trafficking is not affected by PGRN loss.

Previously we showed that PGRN binds to prosaposin (PSAP) to facilitate each other's lysosomal trafficking [30, 51, 52]. PSAP is known to get cleaved in the lysosome to generate saposin peptides, of which saposins A and C are known activators of GCase [46, 53, 54]. This raises



**Fig 3. PGRN deficiency results in decreased GCCase activity in mouse tissues.** GCCase activity was assessed in tissue lysates from 2-month-old WT and *Grn*<sup>-/-</sup> mice, as indicated, using the substrate 4-MU. (n = 5–6, ±SEM, \*p value <0.05, \*\*p-value <0.01, ns, not significant, Student's t-test.

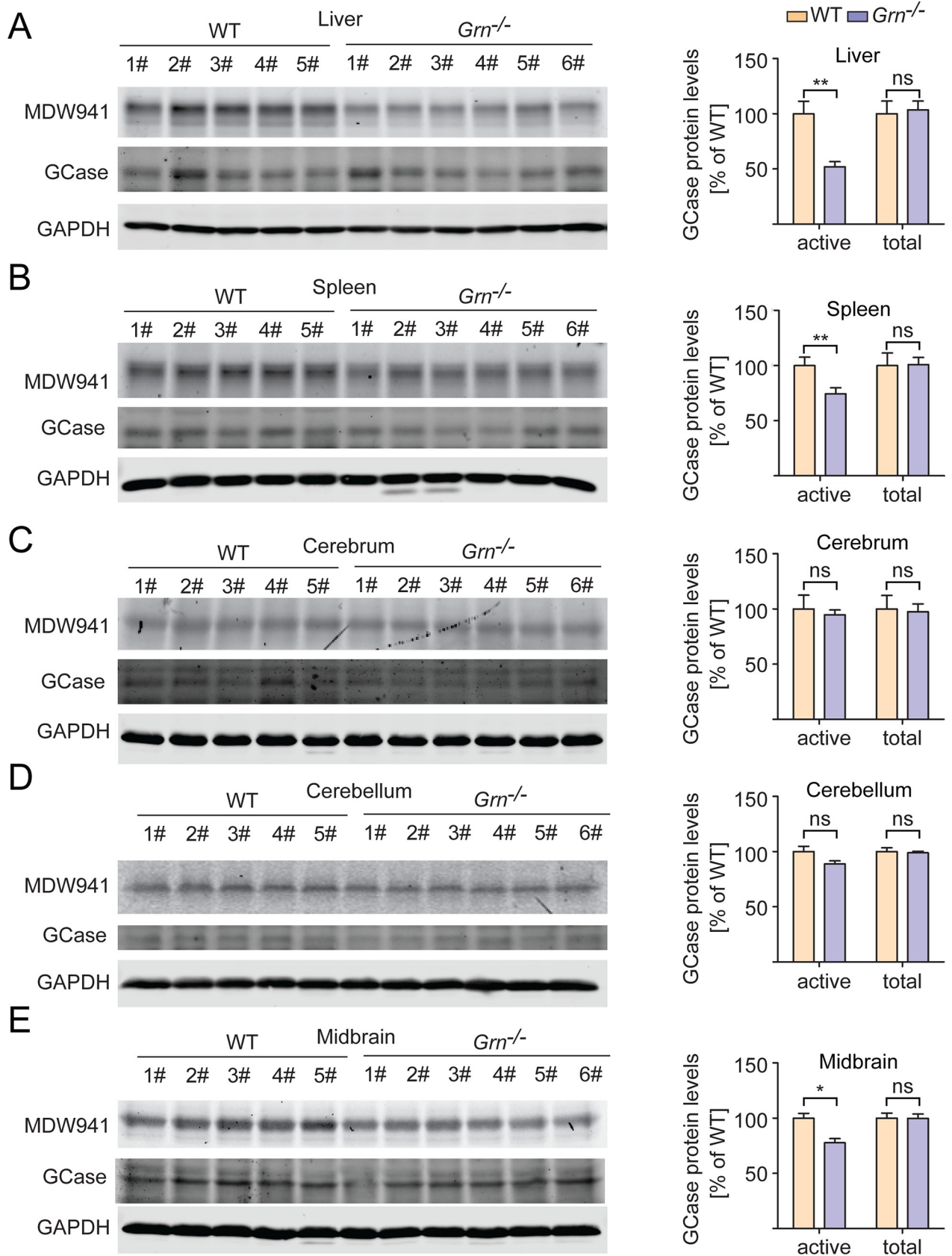
<https://doi.org/10.1371/journal.pone.0212382.g003>

the possibility that the observed decrease in GCCase activity with PGRN loss could be due to a decrease in PSAP or saposin peptides. However, we did not observe a significant change in PSAP or total saposins in the PGRN knockout tissues where GCCase activity was reduced (Fig 6, S2 Table). Similarly, no obvious difference in saposin C levels in WT and PGRN deficient liver lysates were detected following immunoprecipitations using antibodies specific for saposin C (S4 Fig). Unfortunately we cannot test saposin A levels due to a lack of specific antibodies.

With the changes we observed in GCCase activity, and because PGRN and Grn E have been shown to directly activate CTSD *in vitro*, we wanted to test whether PGRN, Grn E, or Grn F could directly augment the activity of GCCase. We performed a 4-MU activity assay of a pharmaceutical grade recombinant GCCase (Cerezyme) with the addition of recombinant His-PGRN, GST-Grn E, GST-Grn F, His-SUMO-saposin C, or GST control. While the addition of saposin C greatly enhanced GCCase activity, such effects were not observed with PGRN or granulin peptides (S5 Fig).

## Discussion

In this study, we demonstrate a physical interaction between the FTLN-related protein, PGRN, and the lysosomal enzyme, GCCase, consistent with previous reports [37, 38]. Additionally, among the granulin peptides, granulin F and to a lesser extent, granulin A, but not the previously reported granulin E, were shown to bind GCCase in the cell lysate (Fig 2A). All the secreted granulins except Grn G show weak binding to GCCase (Fig 2B).

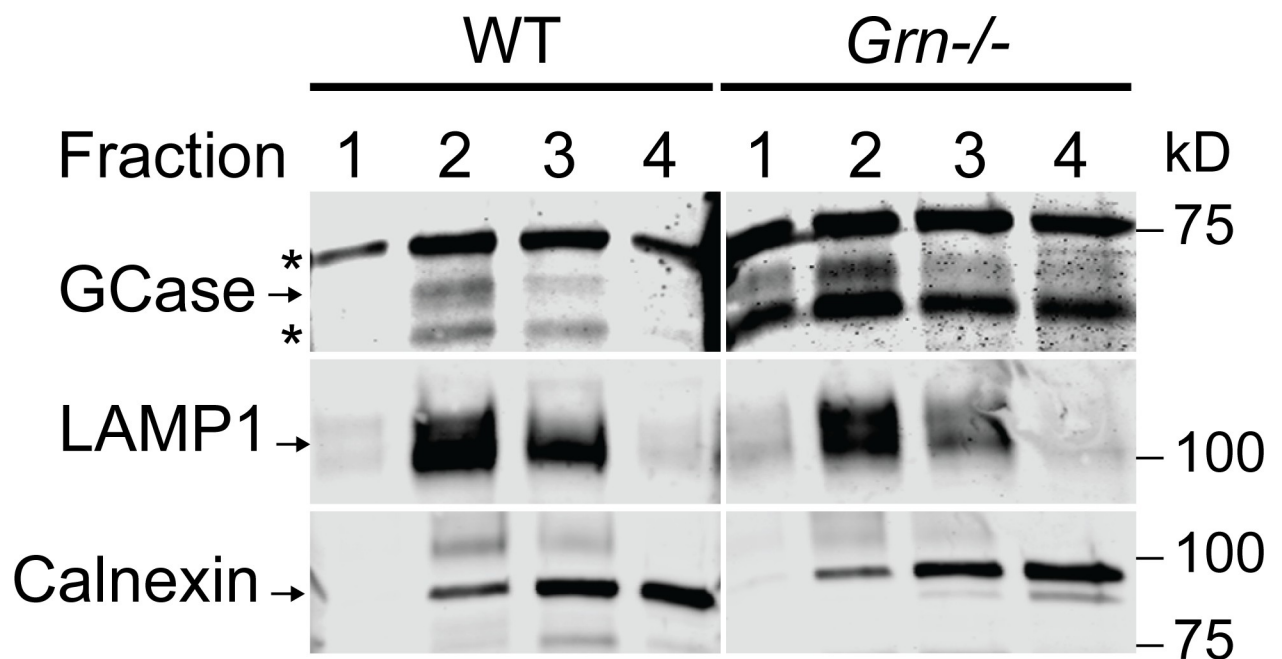


**Fig 4. PGRN deficiency results in decreased GCCase activity without changes in GCCase protein levels.** Tissue lysates from 2-month-old WT and *Grn*<sup>-/-</sup> mice were incubated with the MDW941 GCCase activity probe. The samples were run on SDS-PAGE and MDW941 labeled GCCase (active) was detected using a fluorescent scanner, then western blot was performed to assess total GCCase protein levels. n = 5–6, ±SEM, \*p value <0.05, \*\*p-value <0.01, ns, not significant, Student’s t-test.

<https://doi.org/10.1371/journal.pone.0212382.g004>

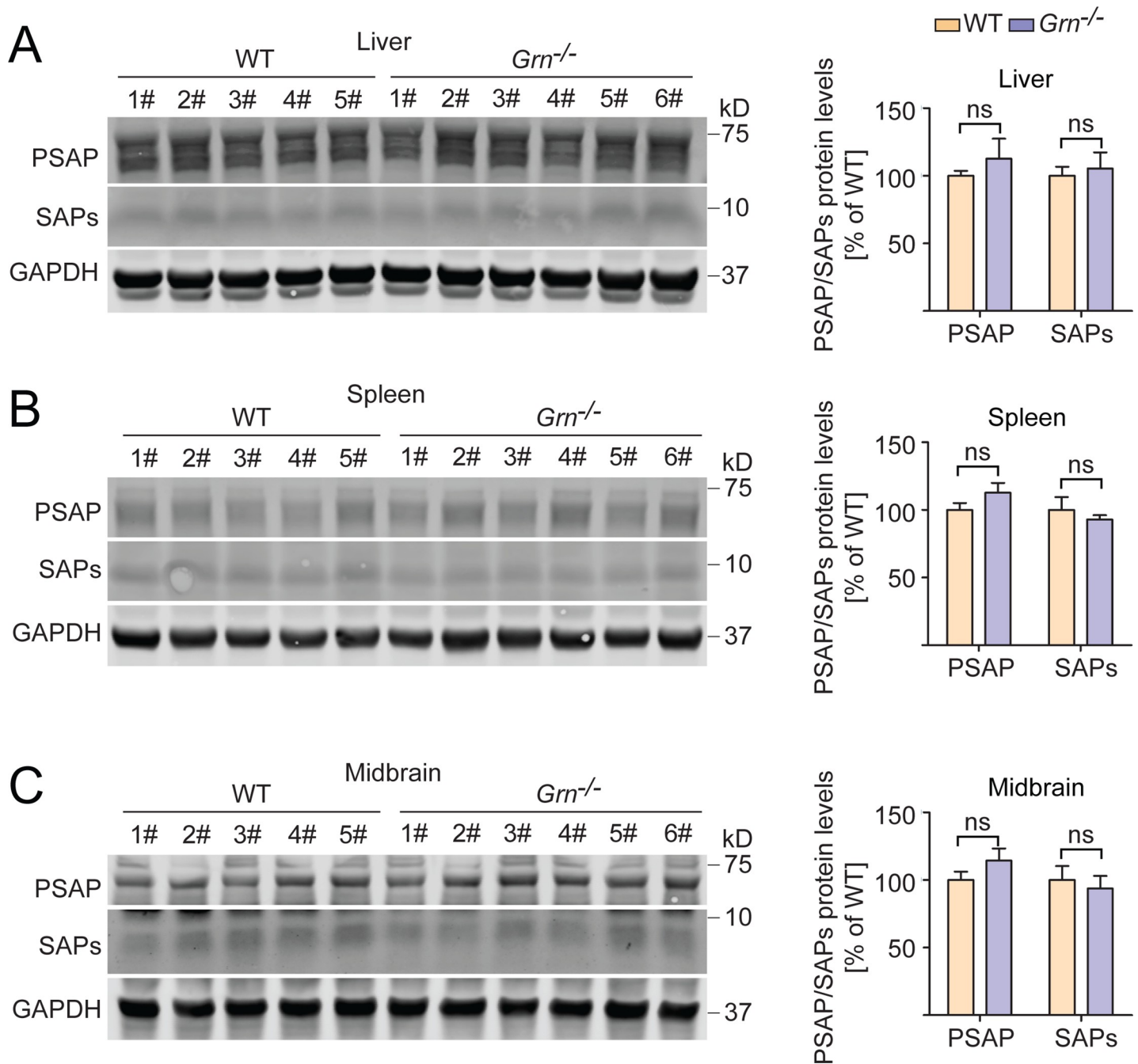
The previous studies examining the relationship between PGRN and GBA primarily utilized a chronic inflammation model based on the administration of ovalbumin (OVA) to WT and *Grn*<sup>-/-</sup> mice over the course of multiple weeks. The authors showed that PGRN interacts with GCCase and acts as a co-chaperone of GCCase and its trafficking receptor, lysosome membrane protein 2 (LIMP-2) [37, 38]. Furthermore, LIMP-2 and GCCase were shown to aggregate in the cytoplasm in PGRN deficient macrophages with experimentally induced chronic lung inflammation models [37, 38]. Unfortunately, we were unable to find a single commercial antibody that specifically recognize the endogenous GCCase protein in immunostaining using WT and *Gba*<sup>-/-</sup> mouse fibroblasts, thus we cannot test whether there is a GCCase trafficking defect with PGRN loss. Nevertheless, MDW41 labeling shows normal localization of active GCCase in PGRN knockout fibroblasts (S6 Fig). Furthermore, GCCase is enriched in the lysosome fraction of *Grn*<sup>-/-</sup> liver similar to that of WT mice (Fig 5), suggesting that the decreased GCCase activity in PGRN deficient liver lysates (Figs 3A and 4A) are not due to defects in GCCase trafficking to lysosomes.

The relationship between PGRN and GCCase is complicated by a series of interrelated factors. Two peptides derived from PSAP, saposin A and saposin C, are known activators of GCCase [46, 53, 54]. We have previously shown that PGRN and PSAP share a lysosomal co-trafficking relationship, wherein PGRN can carry PSAP to the lysosome via the receptor, sortilin, and PSAP can carry PGRN to the lysosome via the cation-independent mannose-6-phosphate receptor (CI-M6PR) or the low-density lipoprotein receptor-related protein 1 (LRP1) [30, 51, 52]. Additionally, PGRN has recently been shown to bind and modulate the activity of the



**Fig 5. GCCase is enriched in the lysosome fraction of WT and *Grn*<sup>-/-</sup> livers.** Lysosomes were isolated from liver tissues of 2-month-old WT and *Grn*<sup>-/-</sup> mice. The presence of GCCase, LAMP1 and calnexin (marker for ER) was determined in the different fractions using Western blot analysis.

<https://doi.org/10.1371/journal.pone.0212382.g005>



**Fig 6. PSAP and total sapsin levels are not changed in PGRN deficient mice.** Mouse tissue lysates from 2-month-old WT and *Grn*<sup>-/-</sup> mice were assessed for PSAP and total sapsin peptide levels, which were normalized to GAPDH. n = 5–6, ±SEM, ns, not significant, Student's t-test.

<https://doi.org/10.1371/journal.pone.0212382.g006>

lysosomal protease, cathepsin D (CTSD), which is the major contributor to proteolytic PSAP processing. Because of these factors, it is possible that PGRN deficiency results in an alteration in PSAP processing and production of sapsin peptides, thereby affecting GCase activation. However, PSAP, total sapsin levels and sapsin C levels do not appear to change in PGRN deficient tissue lysates (Fig 6, S4 Fig), although we cannot rule out the possibility that sapsin A levels might be affected.

We failed to detect any changes in GCase activity with the addition of recombinant PGRN or granulin peptides, but this does not entirely rule out the possibility of a direct activation of GCase by PGRN. Recombinant PGRN and granulins might not fold correctly due to disulfide bond scrambling, or alternatively, further optimization of the conditions for the assay might be required.

Another possibility is that PGRN indirectly affects GCase activity by changing the lysosomal environment. PGRN deficiency has been reported to affect lysosomal pH [55], which could indirectly affect the activity of many lysosomal enzymes. Loss of PGRN also changes lipid contents of lysosomes [56], which could also indirectly affect GCase activity. While the exact mechanism of our findings is currently unknown, and more work needs to be performed to sift through the somewhat muddled relationships of the proteins involved, it is clear that PGRN deficiency leads to reduced GCase activity. This is significant, as lysosomal dysfunction is a commonality between NCL and FTLN with *GRN* mutation. It is possible that the decreased activity of multiple lysosomal hydrolases, including GCase and CTSD, accounts for lysosomal dysfunction with *GRN* mutations.

Mutations and polymorphism in the *GRN* gene have also been associated with Parkinson's disease (PD) [57–60]. Also, parkinsonism is common in *GRN* mutation carriers with FTLN and occurs more frequently than in other forms of FTLN [61]. In addition, reduced serum levels of PGRN were found to be associated with PD risk [62]. Moreover, viral expression of PGRN was shown to protect midbrain dopaminergic neurons and improve the locomotor function in response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment to mimic PD in mouse models [63]. Notably, among different tested brain regions, midbrain, the major PD affected brain region, is the only brain region shows a significant reduction of GCase activity upon PGRN loss (Fig 3, Fig 4). Interestingly, heterozygous mutations in *GBA* is one of the genetic determinants of PD [64, 65]. Our work on the regulation of GCase activity by PGRN might provide a mechanistic explanation underlining the association of PGRN and PD.

## Conclusions

Our data support that PGRN deficiency leads to a reduction of GCase activity *in vivo*.

## Supporting information

**S1 Fig. Physical interaction between mouse PGRN and mouse GCase.** HEK293T cells were transfected with mGBA-FLAG-myc and FLAG-mPGRN constructs as indicated and anti-myc (A) or anti-PGRN (B) immunoprecipitation experiments were carried out. The presence of PGRN and GCase in the immunoprecipitates were detected using Western blot analysis. (TIF)

**S2 Fig. Specificity of mouse anti-GCase antibody for western blot.** A) Primary fibroblasts from WT and *Gba*<sup>-/-</sup> mice. B) Lung tissue lysates from WT and *Gba*<sup>-/-</sup> mice. \* indicates non-specific bands. (TIF)

**S3 Fig. GCase levels in WT and *Grn*<sup>-/-</sup> tissue lysates.** Dashed line indicated where the GCase bands are. (TIF)

**S4 Fig. Levels of saposin C peptides are not changed in PGRN deficient liver lysates.** WT and PGRN-deficient liver lysates were immunoprecipitated using anti-saposin C antibodies

and the IP products were analyzed by Western blot using polyclonal anti-PSAP antibodies. (TIF)

**S5 Fig. Recombinant PGRN, Grn E, or Grn F does not directly increase GCase activity.** (A) Commassie staining of recombinant PGRN, Grn E and Grn F proteins used in the activity assay. (B) Activity of recombinant GCase (Cerezyme) was measured with the addition of recombinant PGRN, Grn E, Grn F, or recombinant saposin C as a positive control. (TIF)

**S6 Fig. MDW941 labeling of active GCase in WT and Grn<sup>-/-</sup> fibroblasts.** WT and Grn<sup>-/-</sup> fibroblasts were labeled for 2 hours with MDW941 before fixation and immunostaining. Scale bar = 20 μm. (TIF)

**S1 Table. List of top 25 SILAC hits from PGRN IP in BV2 cells (heavy: Control cells; light: Grn<sup>-/-</sup> cells).** (XLSX)

**S2 Table. Raw data for Figs 3, 4 & 6.** (XLS)

## Acknowledgments

We would like to thank Dr. Haiyuan Yu for his gifts of cDNA; and Xiaochun Wu for technical assistance. This work is supported by funding to F. Hu from National Institute of Aging and National Institute of Neurological Disorders and Stroke (R01NS095954; R01NS088448) and President's Council of Cornell Women Affinito-Stewart award. The authors declare no additional competing financial interests.

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