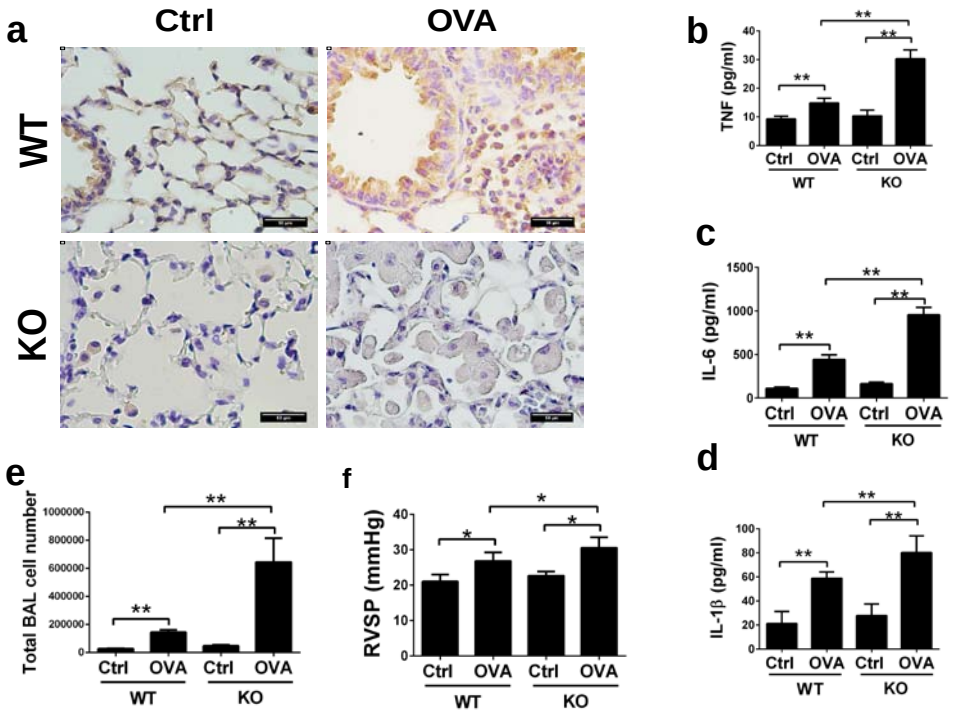
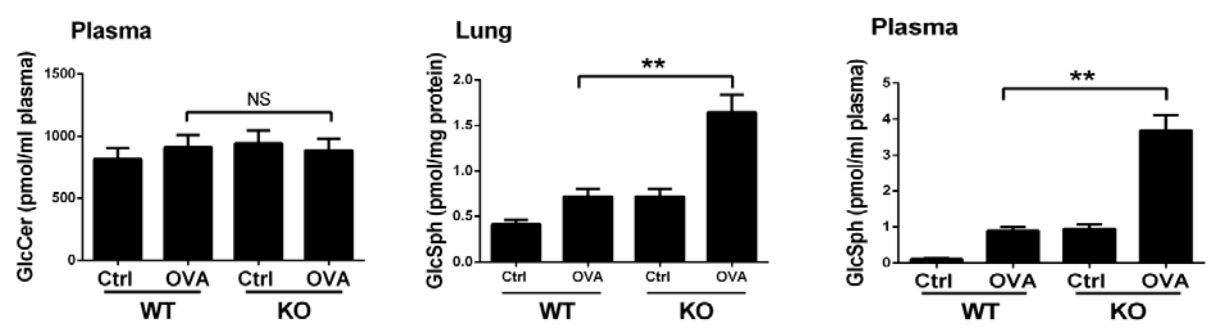
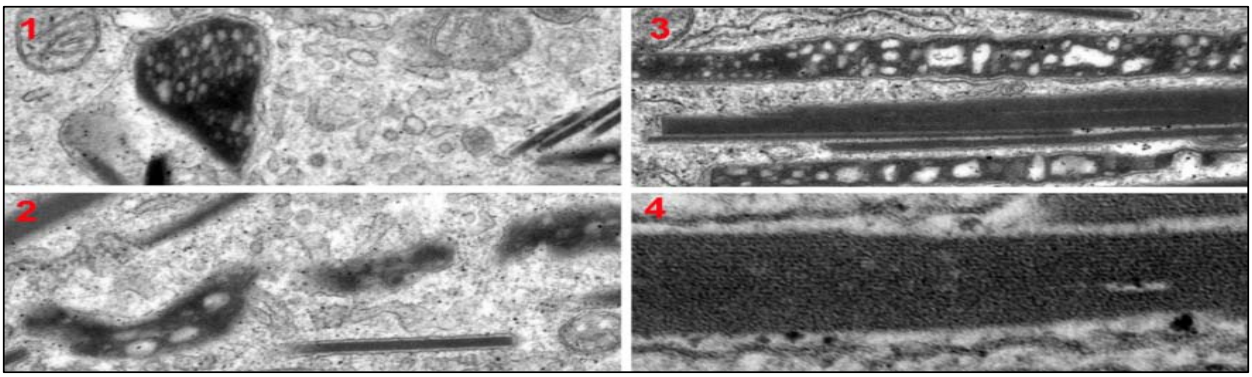
SUPPLEMENTARY DATA



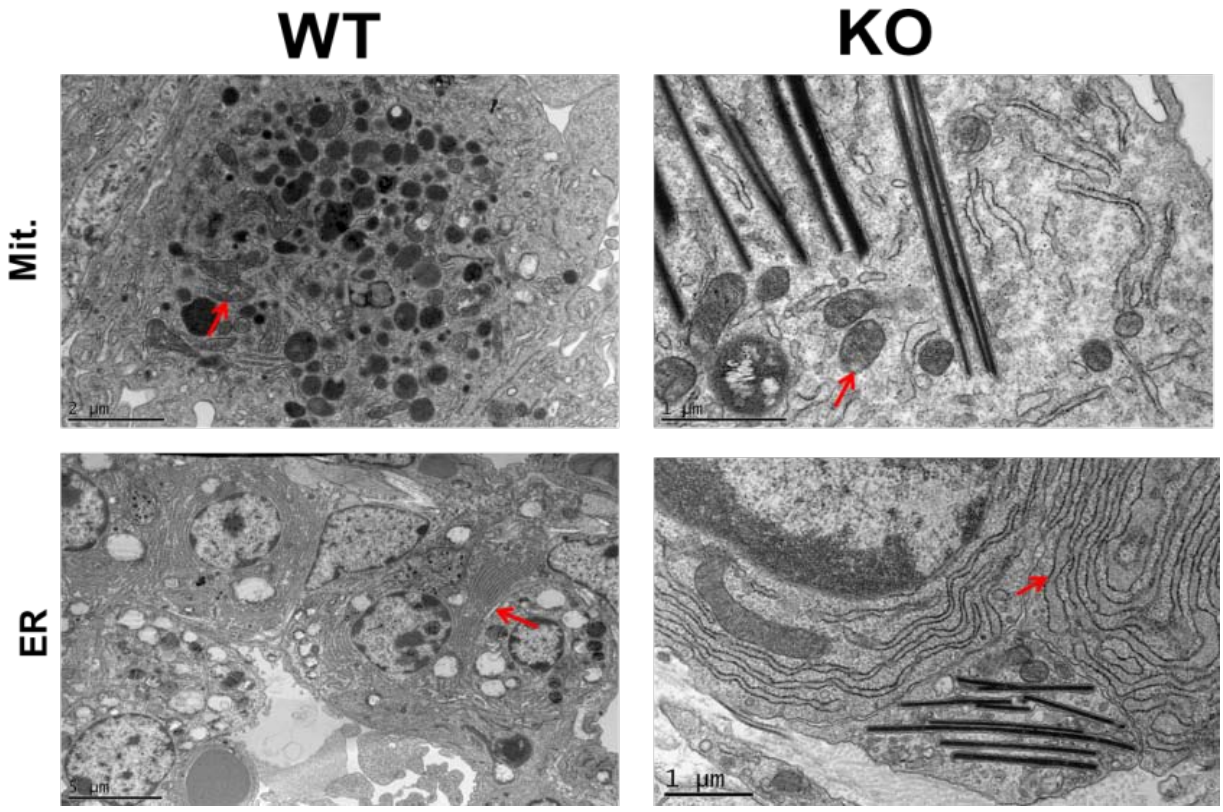
Supplementary Figure S1. PGRN KO mice exhibit more severe inflammation and altered lung function after OVA challenge. **(a)** PGRN expression is markedly induced in WT by OVA challenge. WT and PGRN KO mice were I.P. injected with OVA at Day 1 and Day 15, and starting intranasal challenge with OVA/PBS from Day 29 for three times per week for 4 weeks. The lungs were fixed and processed for embedding into paraffin-blocks. The PGRN expression is measured by immunohistochemistry staining. **(b-f)** WT and PGRN KO mice were challenged with OVA. At the end of experiment, mice underwent catheterization through the right internal jugular vein to right ventricular chamber, and right ventricular systolic pressure was measured. After all the measurements the bronchial alveolar lavage (BAL) was collected for measuring cytokine expressions and cell infiltration. **(b-d)** cytokines in BAL were measured by ELISA kits (n=12). **(e)** cell infiltration in BAL were measured by flowcytometry (n=12). **(f),** RVSP were measured by cauterization(n=6);



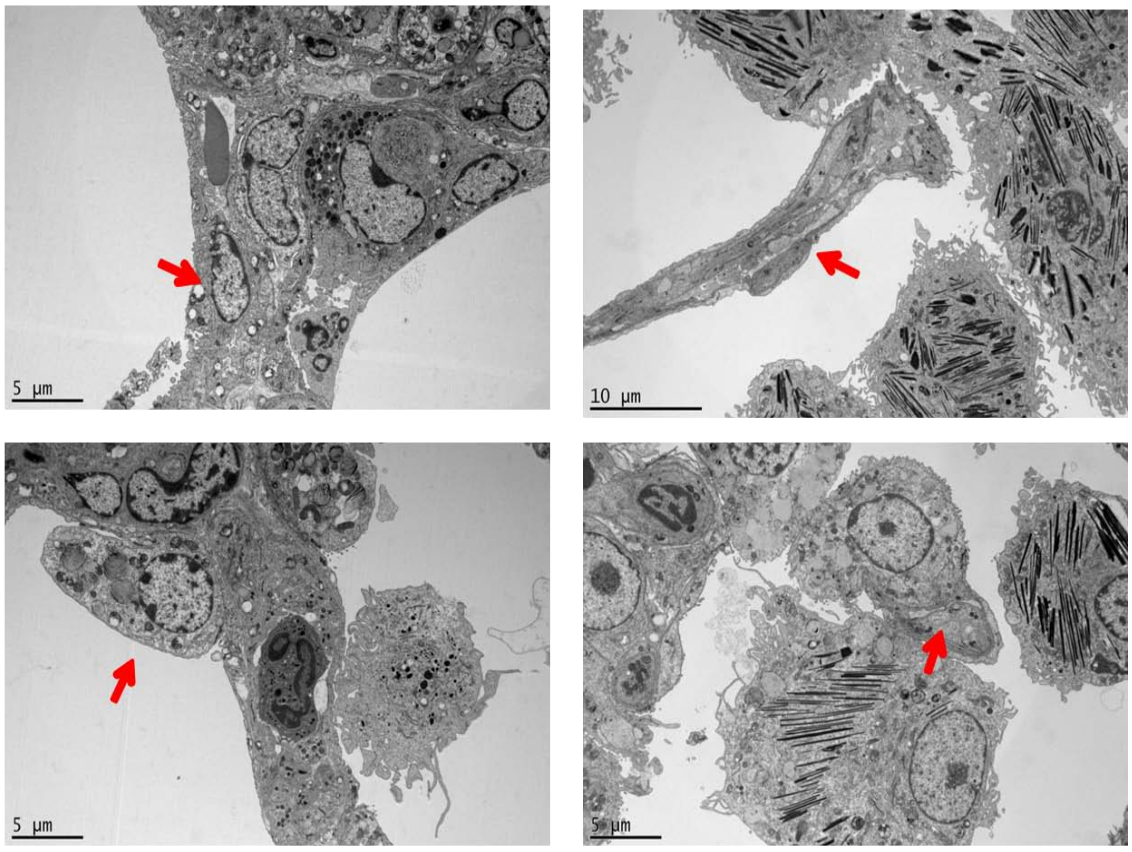
Supplementary Figure S2 Lipid composition analyses in the lung and plasma of PGRN KO mice. WT and PGRN KO mice were challenged with OVA, lung tissues and plasma were collected at the end of experiment. Lipid composition analysis was performed by Lipidomics Core of The Medical University of South Carolina. The levels of GlcCer are not elevated in plasma of OVA-challenged PGRN KO mice compared to the OVA-challenged WT mice (left panel). The levels of GlcSph are elevated in both lung tissues (middle panel) and plasma (right panel) in OVA-challenged PGRN KO mice compare to the OVA-challenged WT mice.



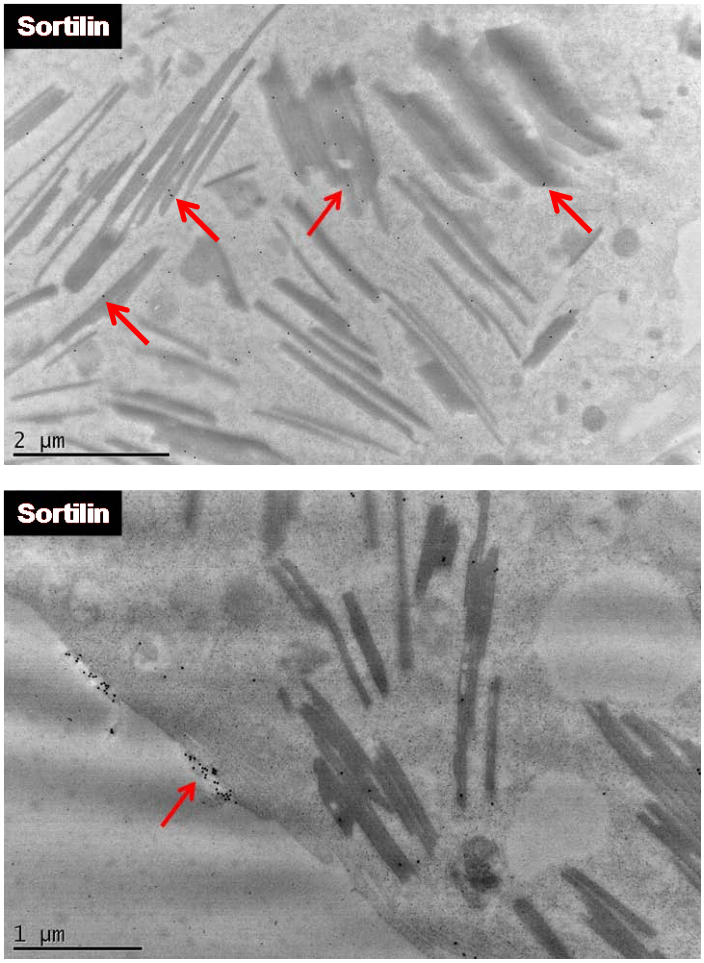
Supplementary Figure S3. Transformation of lysosomes in PGRN null macrophages. 1, Lysosomes show elongated profiles associated with accumulation of material storage (19,500 X); 2, Lysosomes became curved-shape with both high density and low density material in the lysosomes (19,500 X); 3, Lysosome eventually became tubular-like structures with both high density and low density material storage (19,500 X); 4, Low density material was eventually replaced with high density material with intact membrane structure (110,000 X).



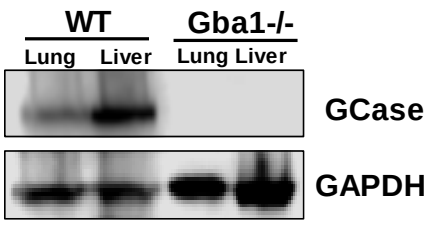
Supplementary Figure S4. Mitochondria and Endoplasmic reticulum were normal in PGRN null macrophages. Lung tissues from OVA-challenged WT and PGRN KO mice were examined under transmission electronic microscope. Mitochondria (Mit.) from WT (11500x) and PGRN KO (31000x), and endoplasmic reticulum (ER) from WT (4400x) and PGRN KO macrophages (7100 X) appeared normal.



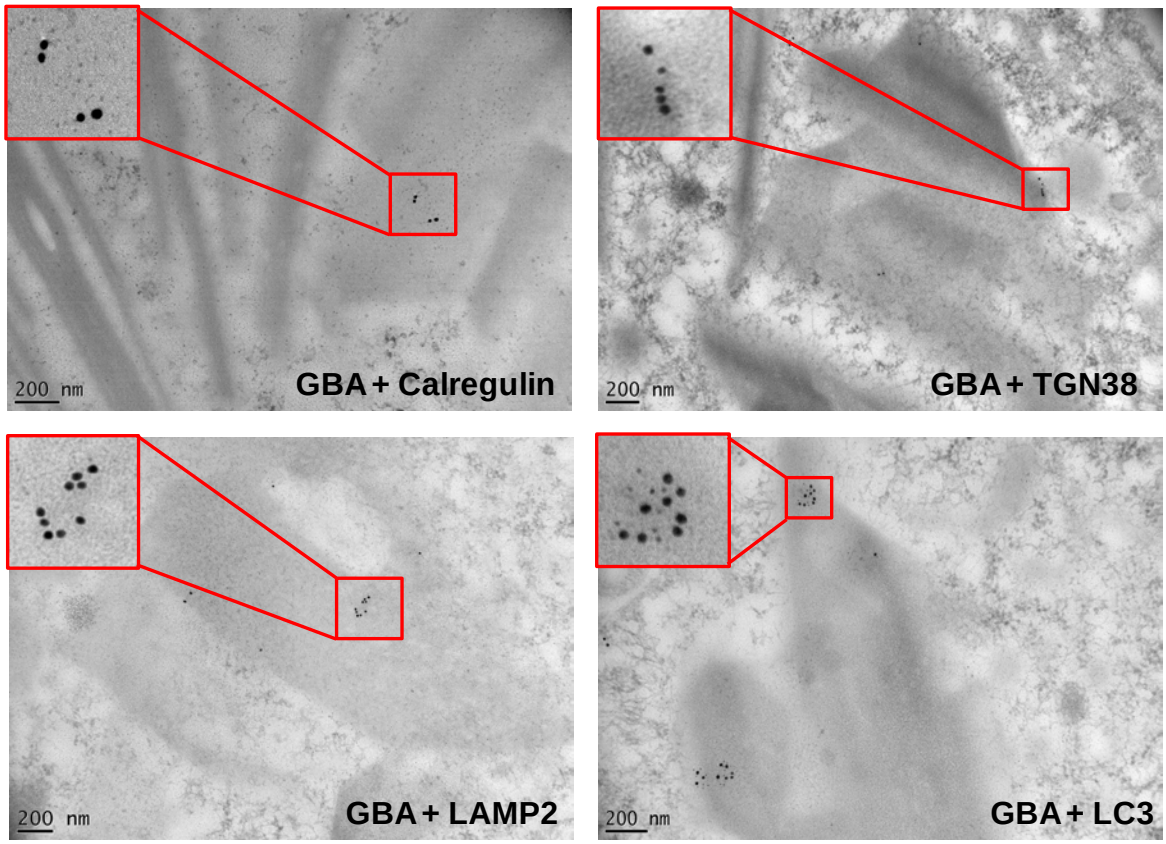
Supplementary Figure S5. Type 1 and 2 pneumocytes were normal in PGRN null mice. Lung tissues from OVA- challenged WT and PGRN KO mice were examined under transmission electronic microscope. Both type 1 and 2 pneumocytes from WT and PGRN KO mice appeared normal (all the images were maginfied 3400x, except type 1 pneumocytes from PGRN KO which were magnified at 2650x).



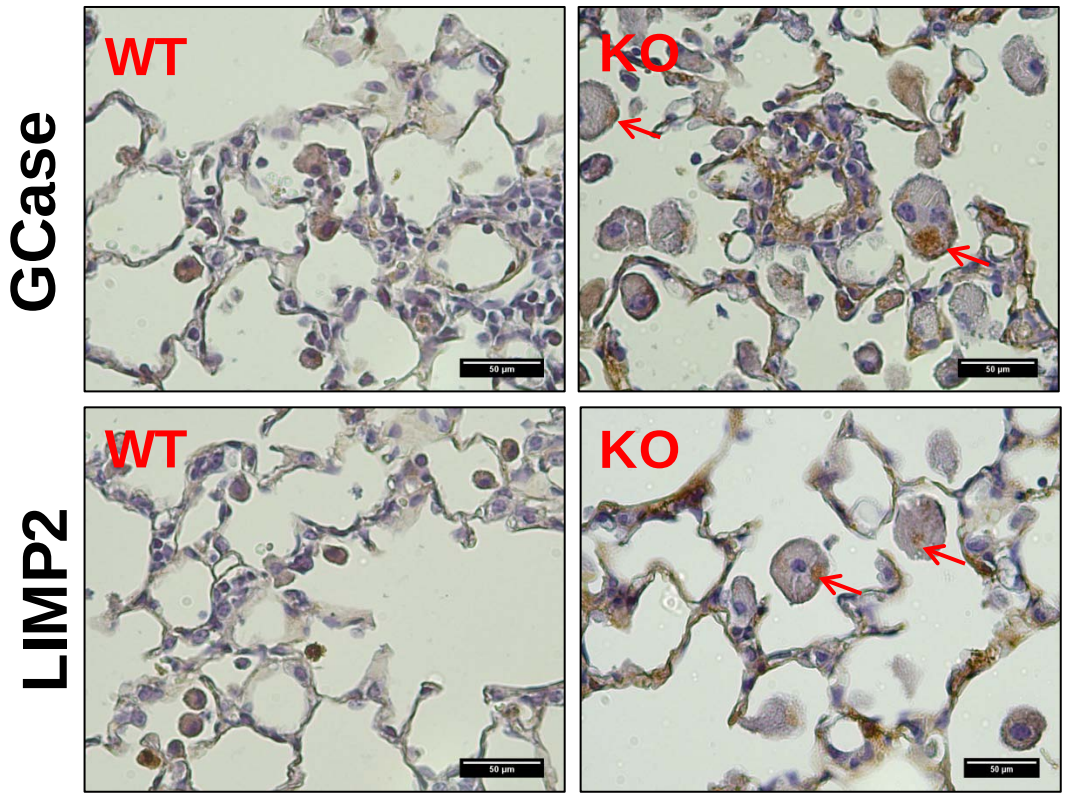
Supplementary Figure S6 Immunogold electronic microscope (IEM) staining of Sortilin in tubular-like lysosome. Lung tissue from PGRN KO mice were stained with Sortilin antibody, and probed with secondary antibody labeled with gold, and observed under EM.



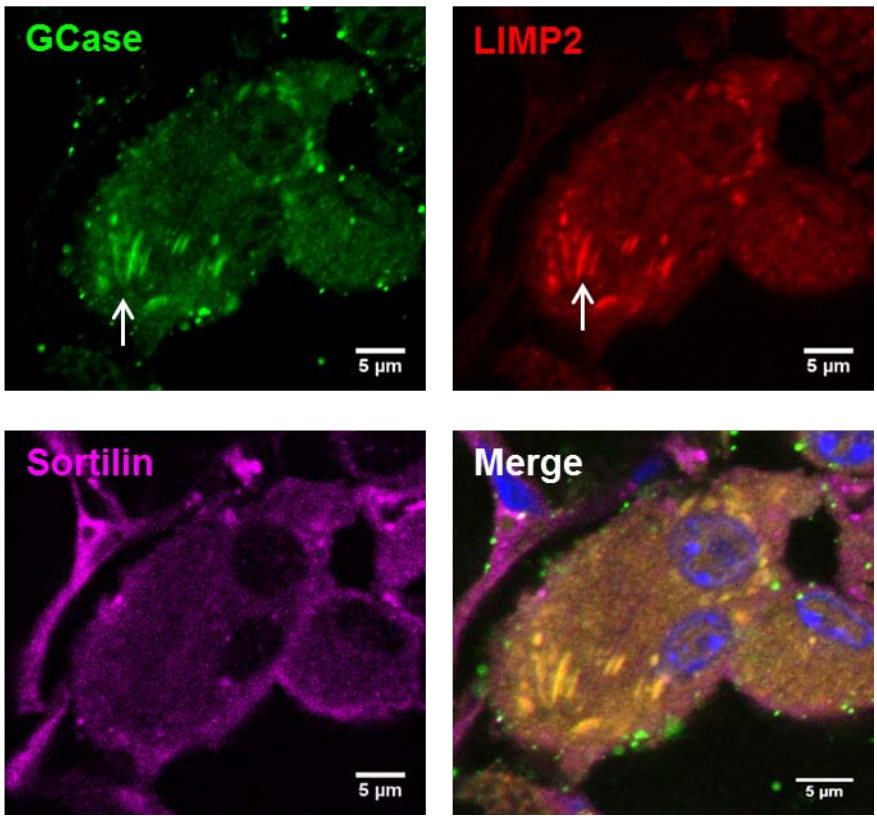
Supplementary Figure S7 Specificity of GCase antibody. Lung and liver tissues from WT and *Gba1*-/- mice were homogenized and further lyzed in RIPA lysis buffer. Protein samples were separated on SDS-PAGE, and the membrane was probed with GCase antibody (SC-100544, from Santa Cruz Biotechnology, Inc).



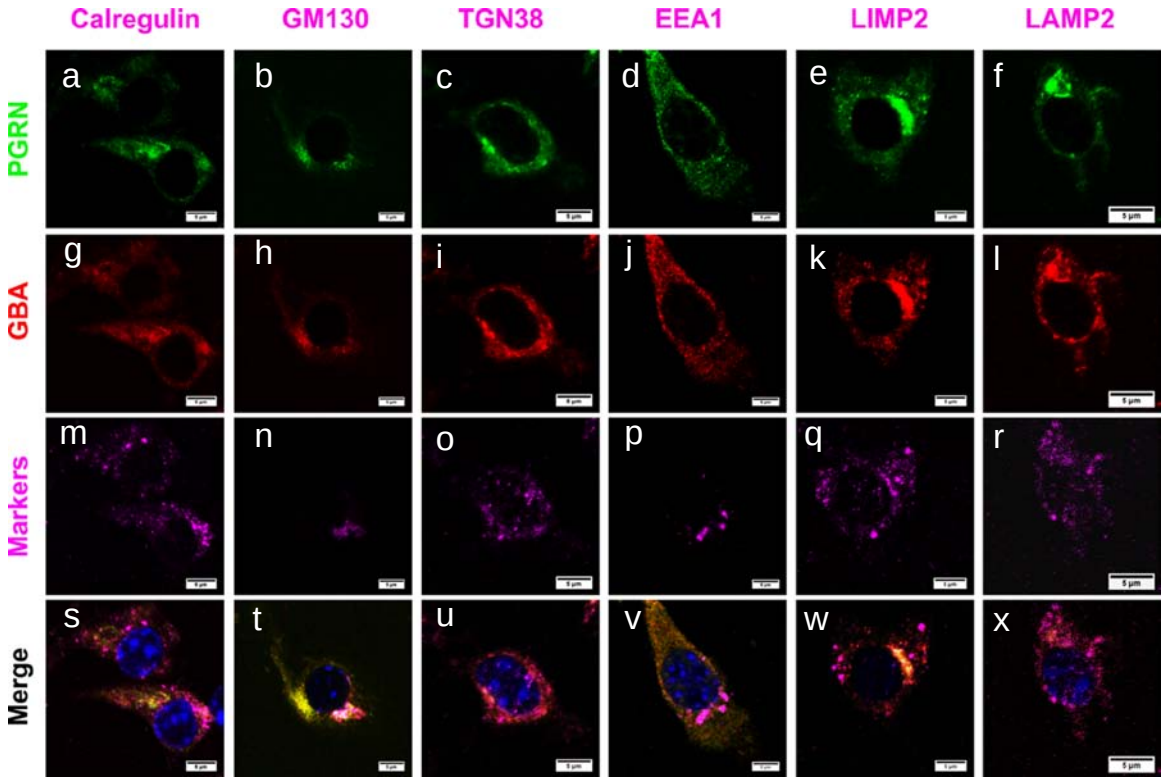
Supplementary Figure S8 Co-immunogold staining of GBA and organelle markers. Lung tissue from PGRN KO mice after OVA challenge were co-immunogold stained with GBA (18 nm particle) and different organelle markers (5 nm particle) including ER marker Calregulin, trans-Golgi marker TGN38, lysosome marker LAMP2 and autophagy marker LC3. These samples were imaged under EM (40,000x).



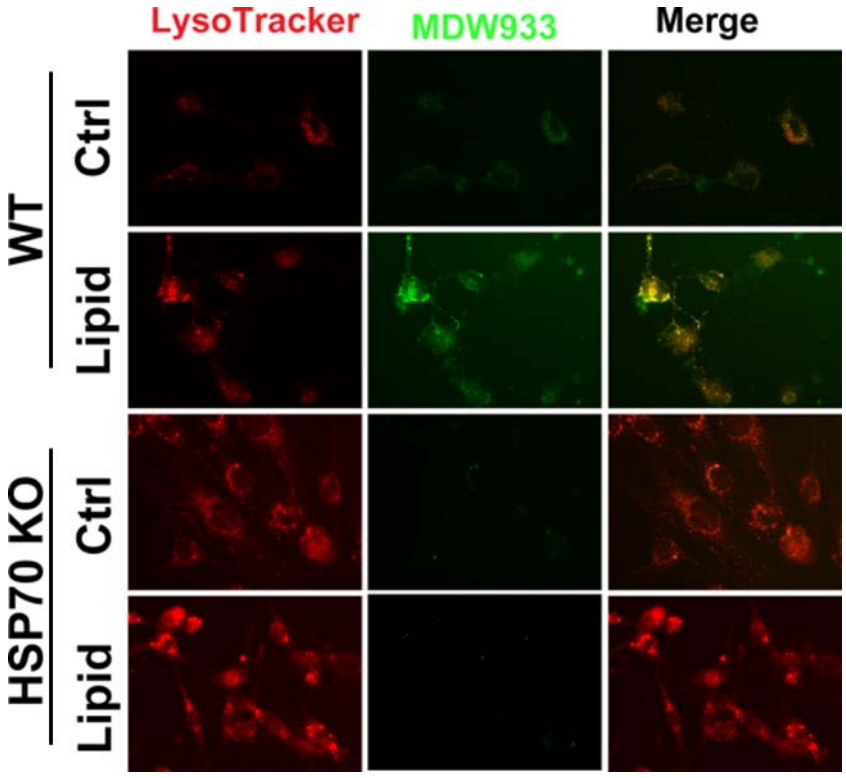
Supplementary Figure S9 GCase and LIMP2 are aggregated in aged PGRN KO mice. Lung tissues from aged PGRN KO mice were stained with antibodies against GCase and LIMP2 by IHC staining. The aggregates of GCase and LIMP2 are indicated by red arrows.



Supplementary Figure S10 GCase and LIMP2 are aggregated in PGRN KO mice. Frozen section of lung tissue from PGRN KO mice challenged with OVA were immunofluorescence stained with antibody against GCase, LIMP2, and Sortilin respectively. The aggregates of GCase and LIMP2 are indicated by white arrow.



Supplementary Figure S11 Co-localization of GCase and PGRN in the intracellular trafficking compartments in macrophages. BMDMs from WT mice were fixed and the expressions of PGRN and GCase, as well as the markers for the trafficking compartments were detected with respective specific primary antibodies, followed by corresponding secondary antibodies labeled with different fluorescence dyes. PGRN was stained with sheep anti-mouse PGRN primary antibody and secondary antibody labeled with Alexa-488 (**a-f**), GCase was stained with rabbit anti-mouse primary antibody, followed by Cy3-labeled secondary antibody (**g-l**), and the markers for the trafficking compartments (Calregulin, GM130, TGN38, EEA1, LIMP2, and LAMP2) were stained with corresponding mouse-originated primary antibodies, and followed by Alexa-647 labeled secondary antibodies (**m-r**). Panels **s-x** are the merged images of 4-color staining. The nucleus was stained with DAPI (blue).



Supplementary Figure S12 Live image of co-localization of MDW933 with LysoTracker in WT and HSP70 KO cells. WT and HSP70 KO lung epithelium cells were cultured in Lab-Tek Chamber cover glass system, and stimulated with or without lipid lysis for 24 hours. MDW933 (50nM) and LysoTracker (100 nM) were added to the medium for 1h. The images were taken by Applied Precision Personal DV live-cell imaging system at NYU core facility.

Table S1 List of genes that may be PGRN-dependent GCase associated proteins

1	Perlecan (Heparan sulfate proteoglycan 2) OS=Mus musculus GN=Hspg2 PE=4 SV=1 - [B1B0C7_MOUSE]
	Moesin OS=Mus musculus GN=Msn PE=1 SV=3 - [MOES_MOUSE]
2	T-complex protein 1 subunit zeta OS=Mus musculus GN=Cct6a PE=1 SV=3 - [TCPZ_MOUSE]
2	Heat shock 70 kDa protein 1a OS=Mus musculus GN=Hspa1a PE=1 SV=1 - [HSPA1A_MOUSE]
	Uncharacterized protein OS=Mus musculus GN=Gm8991 PE=4 SV=1 - [E9Q7H5_MOUSE]
	EH domain-containing protein 4 OS=Mus musculus GN=Ehd4 PE=1 SV=1 - [EHD4_MOUSE]
	Polymeric immunoglobulin receptor OS=Mus musculus GN=Pigr PE=1 SV=1 - [PIGR_MOUSE]
1	Leukocyte elastase inhibitor A OS=Mus musculus GN=Serpinb1a PE=1 SV=1 - [ILEUA_MOUSE]
	Uncharacterized protein (Fragment) OS=Mus musculus GN=Fus PE=4 SV=1 - [G3UXT7_MOUSE]
	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2 - [A1AT2_MOUSE]
	Protein S100-A9 OS=Mus musculus GN=S100a9 PE=1 SV=3 - [S10A9_MOUSE]
	Vinculin OS=Mus musculus GN=Vcl PE=1 SV=4 - [VINC_MOUSE]
	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1 - [CLUS_MOUSE]
	Putative ATP-dependent RNA helicase Pl10 OS=Mus musculus GN=D1Pas1 PE=1 SV=1 - [DDX3L_MOUSE]
	Niban-like protein 1 OS=Mus musculus GN=Fam129b PE=1 SV=2 - [NIBL1_MOUSE]
	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1 - [TRFE_MOUSE]
	Annexin A11 OS=Mus musculus GN=Anxa11 PE=1 SV=2 - [ANX11_MOUSE]
	Ankycorbin OS=Mus musculus GN=Rai14 PE=1 SV=1 - [RAI14_MOUSE]
	Ehd2 protein OS=Mus musculus GN=Ehd2 PE=2 SV=1 - [Q8R2X0_MOUSE]
	Peroxiredoxin 1 (Fragment) OS=Mus musculus GN=Prdx1 PE=4 SV=1 - [B1AXW5_MOUSE]
	LIM and SH3 protein 1 (Fragment) OS=Mus musculus GN=Lasp1 PE=4 SV=1 - [A2A6G9_MOUSE]
	Ribonuclease inhibitor OS=Mus musculus GN=Rnh1 PE=1 SV=1 - [RINI_MOUSE]
	Heterogeneous nuclear ribonucleoprotein U, isoform CRA_b OS=Mus musculus GN=Hnrnpu PE=4 SV=1 - [G3XA10_MOUSE]
	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1 - [FIBB_MOUSE]
	NK13 OS=Mus musculus GN=Serpinb6b PE=2 SV=2 - [O08804_MOUSE]
	Chloride intracellular channel protein 4 OS=Mus musculus GN=Clic4 PE=1 SV=3 - [CLIC4_MOUSE]
	Dimethylaniline monooxygenase [N-oxide-forming] 2 OS=Mus musculus GN=Fmo2 PE=1 SV=3 - [FMO2_MOUSE]
	Ceruloplasmin, isoform CRA_f OS=Mus musculus GN=Cp PE=4 SV=1 - [G3X9T8_MOUSE]
	Myosin light polypeptide 6 OS=Mus musculus GN=Myl6 PE=1 SV=3 - [MYL6_MOUSE]
	Fibrinogen, alpha polypeptide OS=Mus musculus GN=Fga PE=2 SV=1 - [Q99K47_MOUSE]
	Alcohol dehydrogenase 1 OS=Mus musculus GN=Adh1 PE=2 SV=2 - [ADH1_MOUSE]
	Isoform 4 of Myosin-XVIIIa OS=Mus musculus GN=Myo18a - [MY18A_MOUSE]
	Copine-3 OS=Mus musculus GN=Cpne3 PE=1 SV=2 - [CPNE3_MOUSE]
	Pulmonary surfactant-associated protein D OS=Mus musculus GN=Sftpd PE=2 SV=1 - [SFTPD_MOUSE]
	Actin-related protein 2/3 complex subunit 1B OS=Mus musculus GN=Arpc1b PE=1 SV=4 - [ARC1B_MOUSE]
	F-actin-capping protein subunit alpha-1 OS=Mus musculus GN=Capza1 PE=1 SV=4 - [CAZA1_MOUSE]
	Integrin beta OS=Mus musculus GN=Itgb2 PE=2 SV=1 - [Q542I8_MOUSE]
	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=2 SV=1 - [FIBG_MOUSE]
	Long-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus GN=Acadl PE=2 SV=2 - [ACADL_MOUSE]

Note: 1 known PGRN binding proteins, 2 HSP70 and its co-chaperones.