Zhou et al., http://www.jcb.org/cgi/content/full/jcb.201502029/DC1

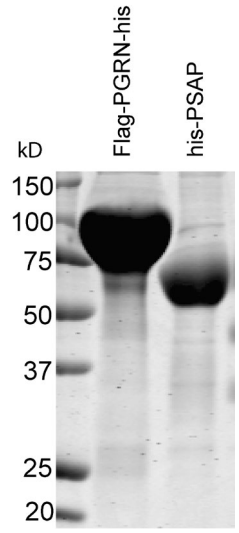

Figure S1. **Coomassie staining of SDS-PAGE with purified Flag-PGRN-his and PSAP proteins used in this study.** Conditioned media were collected from HEK293T cells expressing Flag-PGRN-his or his-PSAP and incubated with cobalt beads. Proteins were eluted with imidazole and buffers were changed to PBS using the Centricon device.

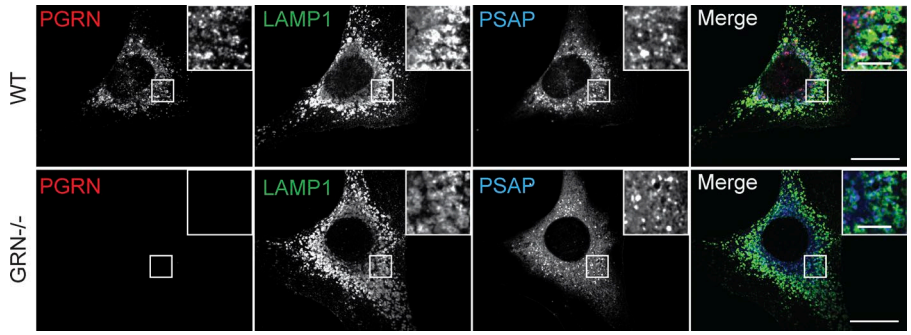

Figure S2. **PGRN is not required for PSAP lysosomal targeting in fibroblasts.** Immunostaining for PGRN, LAMP1, and PSAP in fibroblasts derived from wild-type and PGRN$^{-/-}$ mice using sheep anti–mouse PGRN, rat anti–mouse LAMP1, and rabbit anti–mouse PSAP antibodies. Representative images from three replicated experiments are shown. Bars: (main) 20 µm; (inset) 5 µm.

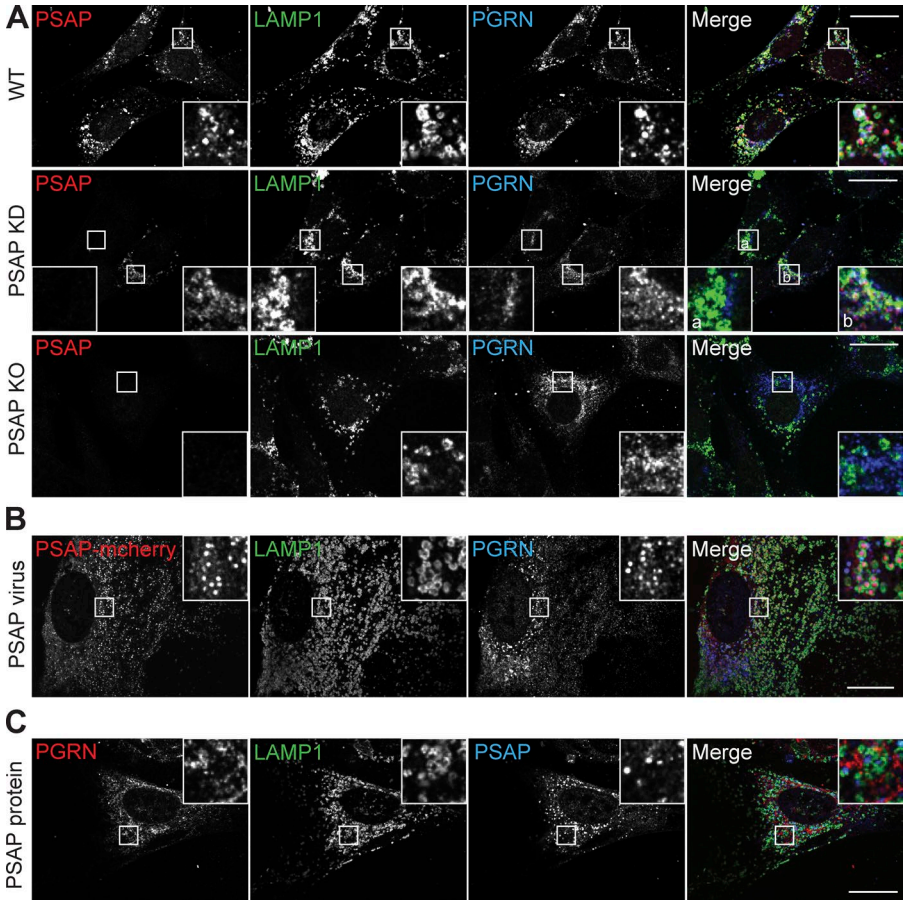

Figure S3. **Mislocalization of PGRN in fibroblasts with PSAP expression knocked down by shRNA or ablated with CRISPR.** (A) Immortalized wild-type fibroblasts were infected with retroviruses expressing shRNAs against mPSAP or pLentiCRISPR viruses expressing guide RNAs targeted to mPSAP. Cells were selected with puromycin and stained with sheep anti-PGRN, rat anti-LAMP1, and rabbit anti-PSAP antibodies. In PSAP knockdown samples, two cells with or without visible PSAP expression were shown. (B) Expression of mCherry-PSAP fully rescues the PGRN localization defect in PSAP$^{-/-}$ fibroblasts. PSAP$^{-/-}$ fibroblasts were infected with lentiviruses expressing mCherry-PSAP and stained with sheep anti-PGRN and rat anti-LAMP1 antibodies. (C) PSAP$^{-/-}$ fibroblasts were treated with purified PSAP (5 µg/ml) for 12 h. PSAP and PGRN localization was examined as in B. Representative images from three replicated experiments are shown. Bars: (main) 20 µm; (inset) 5 µm.

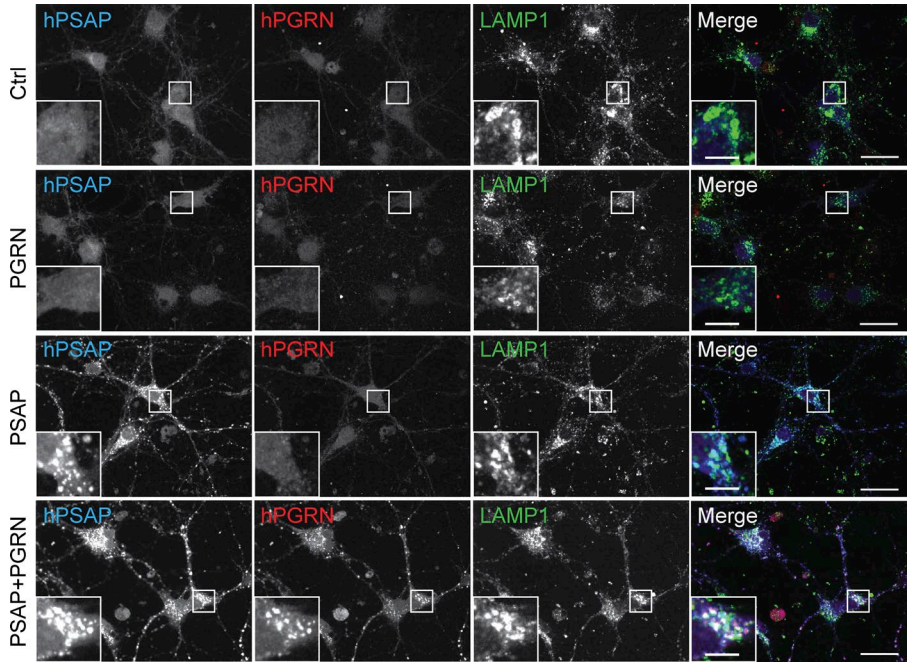

Figure S4. **PSAP facilitates PGRN lysosomal targeting from the extracellular space in neurons.** Recombinant human PSAP and human PGRN were added to primary cortical neurons cultured from newborn PGRN$^{-/-}$ mice at a concentration of 5 µg/ml. After 12 h, cells were washed with PBS and fixed and stained with antibodies specific for human PGRN, mouse LAMP1, and human saposin B (PSAP) proteins. Representative images from three replicated experiments are shown. Bars: (main) 20 µm; (inset) 5 µm.

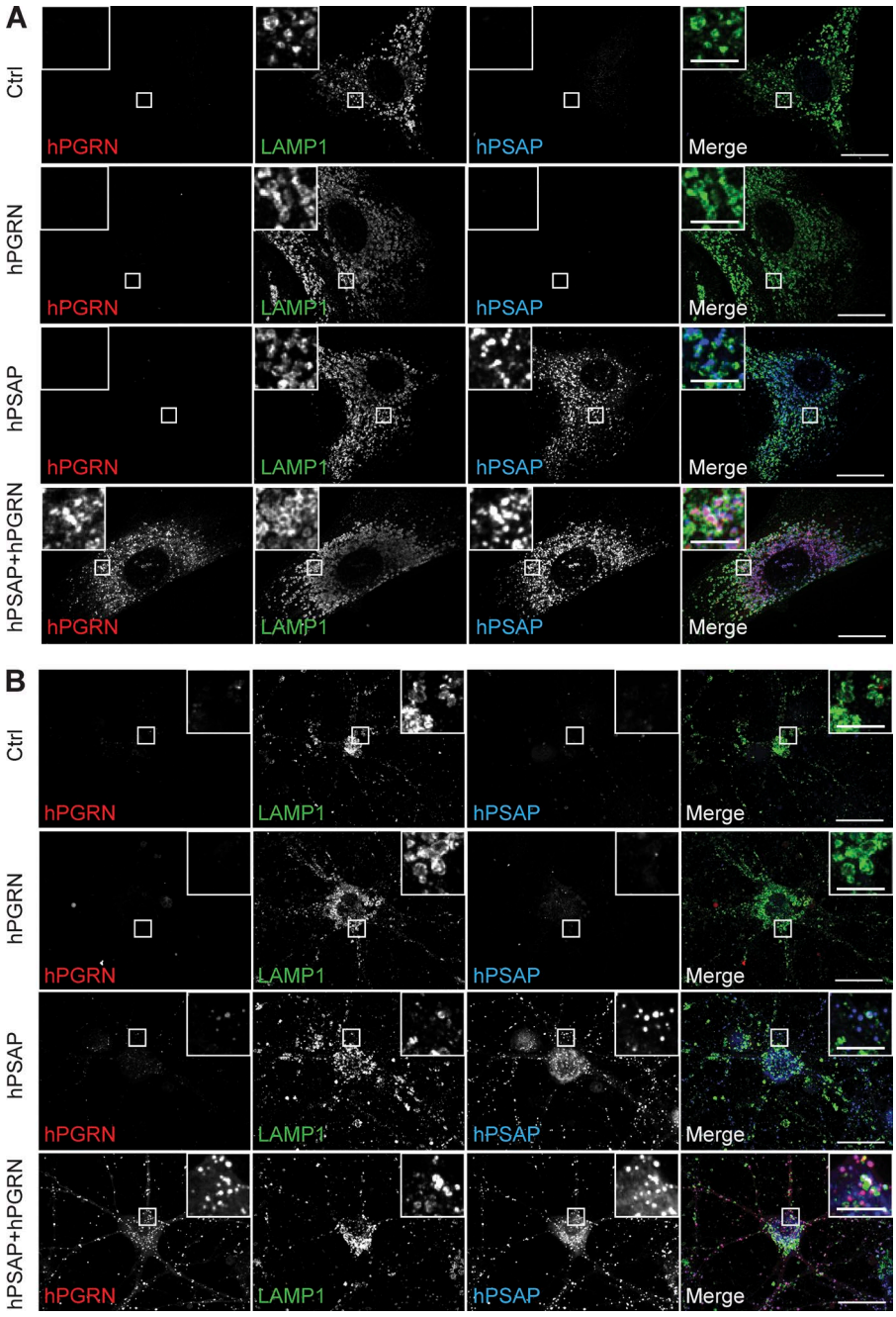

Figure S5. **PSAP facilitates PGRN lysosomal targeting from the extracellular space in Sort1$^{-/-}$ fibroblasts and neurons.** (A and B) Recombinant human PSAP and human PGRN were added to Sort1$^{-/-}$ fibroblasts (A) and cortical neurons (B) at a concentration of 5 µg/ml in serum-free medium. After 12 h, cells were washed with PBS and fixed and stained with antibodies specific for human PGRN, mouse LAMP1, and human saposin B (PSAP) proteins. Representative images from three replicated experiments are shown. Bars: (main) 20 µm; (inset) 5 µm.

Tables S1 and S2 lists SILAC hits identified with GFP-PGRN in T98G cells and with FLAG-PSAP pull-down in fibroblasts, respectively.