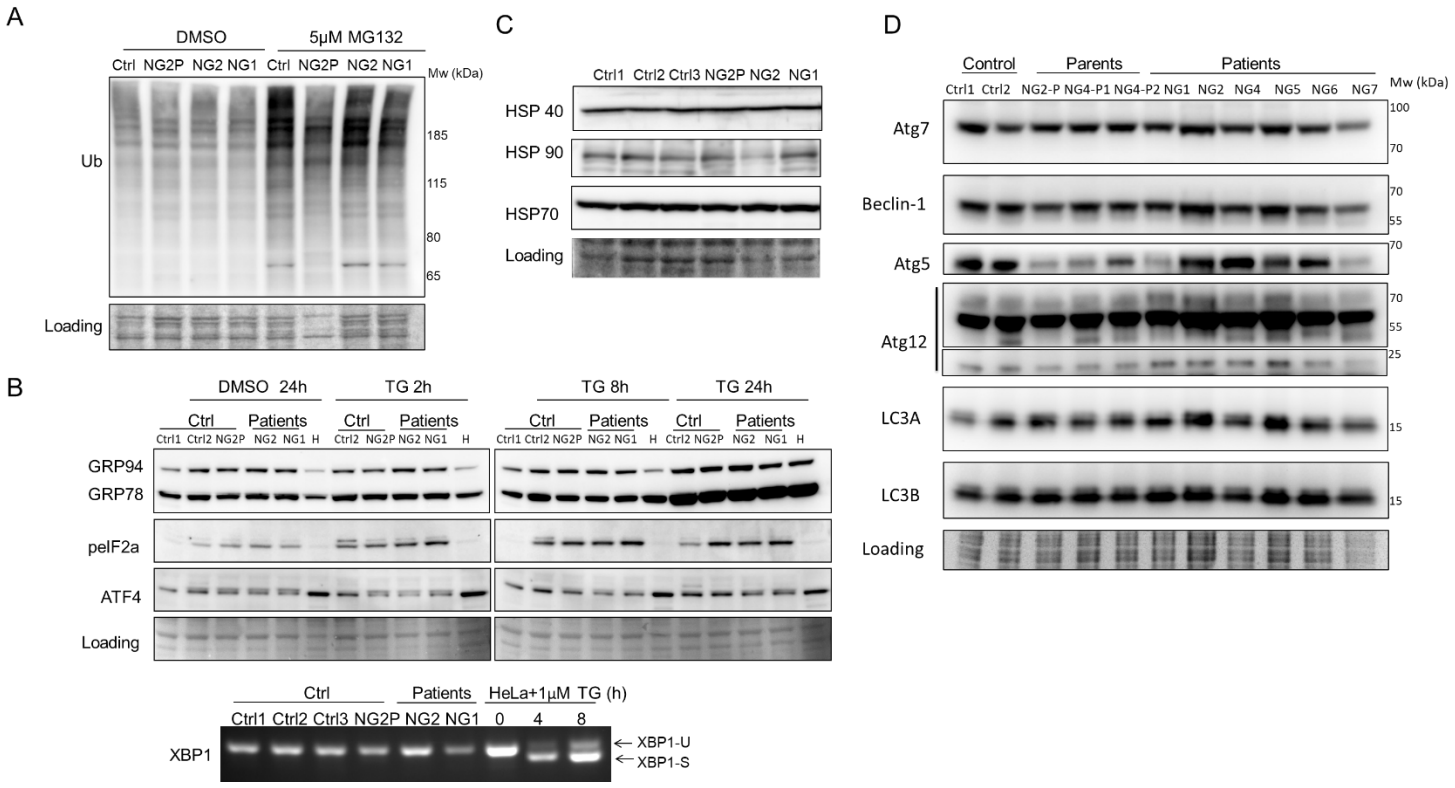

Figure S1. No abnormalities of ubiquitination (A), unfolded protein response (UPR) signal transduction (B), proteostasis (C) or autophagy (D) were found in NGLY1 deficient fibroblasts. (A) The human skin fibroblasts derived from control and NGLY1 patients were treated with 5μM MG132 or DMSO for 16h followed by Western blot analysis of ubquitin. (B) Fibroblasts and HeLa cells were treated with 1μM thapsgargin (TG) or DMSO for different durations as indicated followed by Western blot (upper panel) and RT-PCR (lower panel) analysis. (C)WB analysis of proteostasis indicators in human fibroblasts. (D) WB analysis of autophagy markers in human fibroblasts. H: HeLa; TG: thapsgargin; Ctrl: control fibroblast; NG: NGLY1 patient fibroblast; NG-P (1/2): NGLY1 patient parent's fibroblast (1, father; 2, mother).

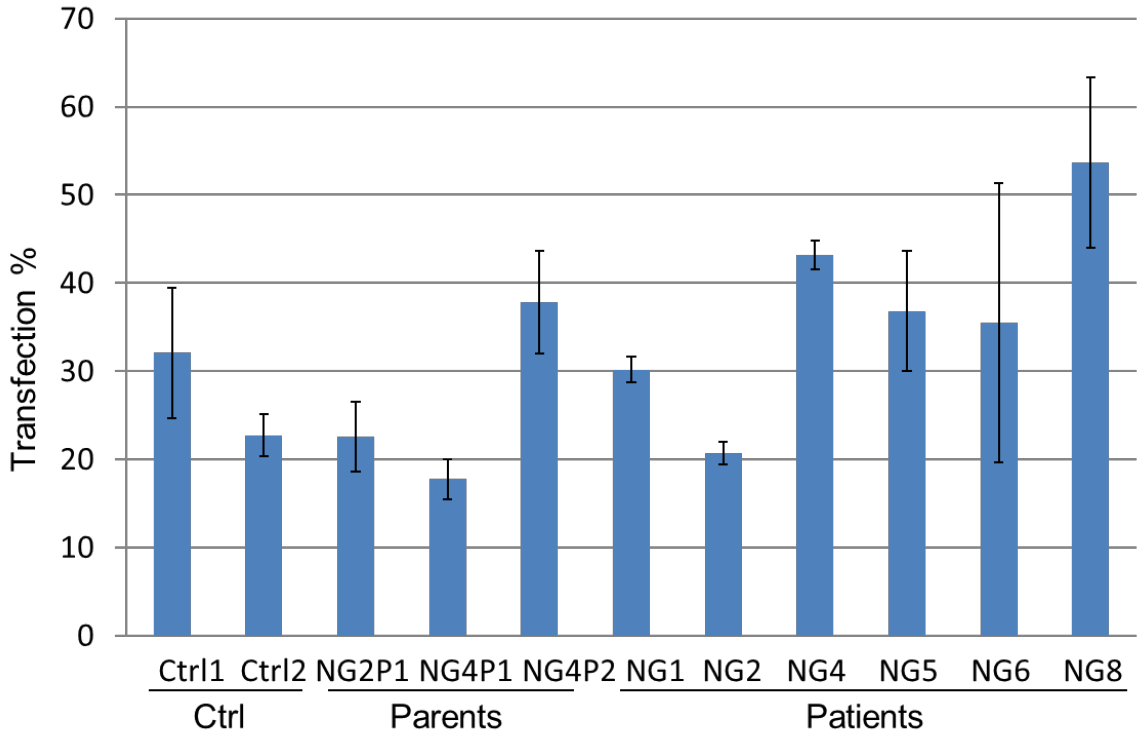

Figure S2. Transfection efficiency by electroporation across fibroblasts derived from individuals. Cell were electroporated with pmaxGFP™ Vector. The expressed GFP was observed under microscope to calculate the transfection efficiency as percentage of GFP positive cells. NG-P (1/2): NGLY1 patient parent's fibroblast (1, father; 2, mother).

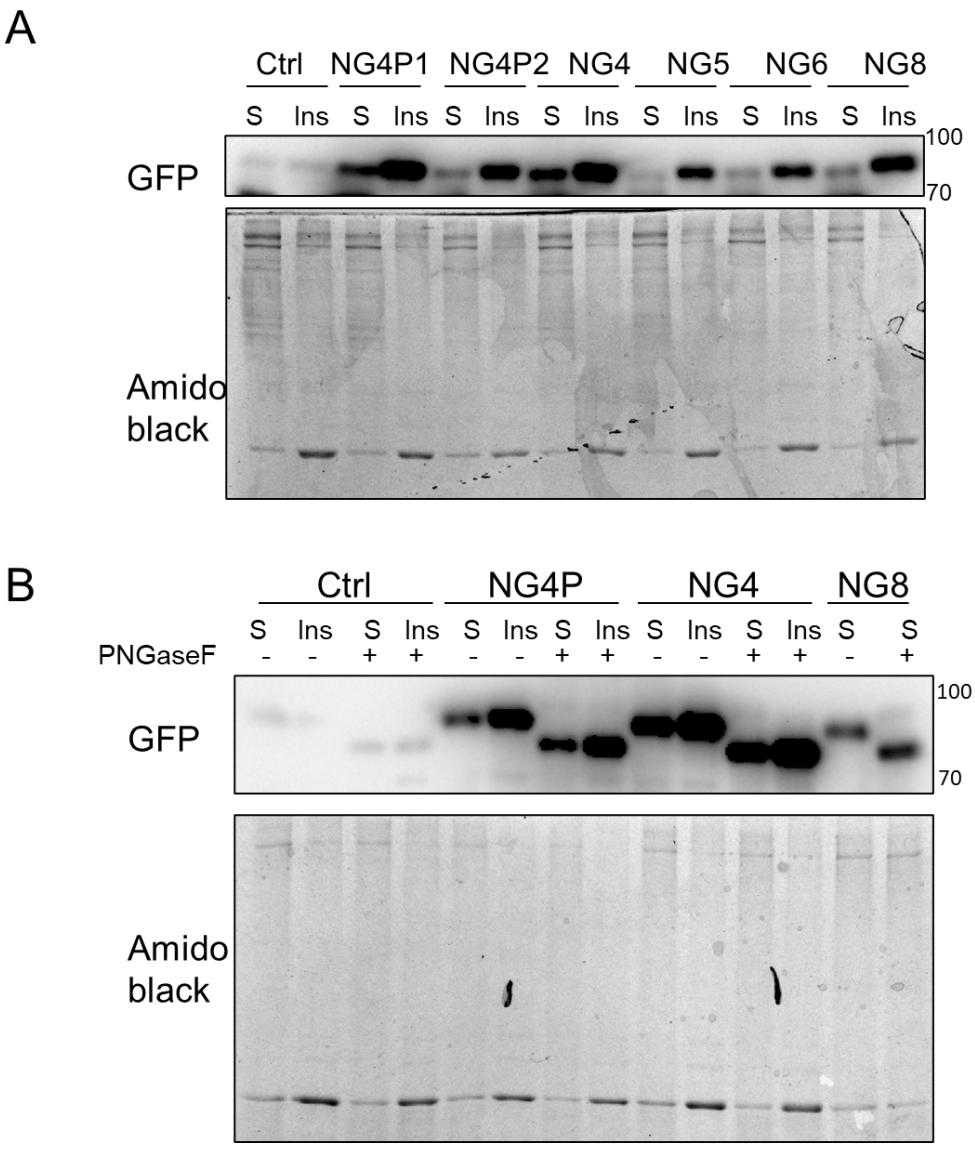

Figure S3. NGLY1 deficiency caused insoluble accumulation of transfected A1AT-NHK-GFP substrates (related to Figure 1). A1AT-NHK-GFP substrates were electroporated followed by WB analysis of soluble (S) and insoluble (Ins) protein without (A) or with (B) PNGase F treatment in each sample. Amido black stain was used as loading control. Ctrl: control fibroblast; NG: NGLY1 patient fibroblast; NG-P (1/2): NGLY1 patient parent's fibroblast (1, father; 2, mother).

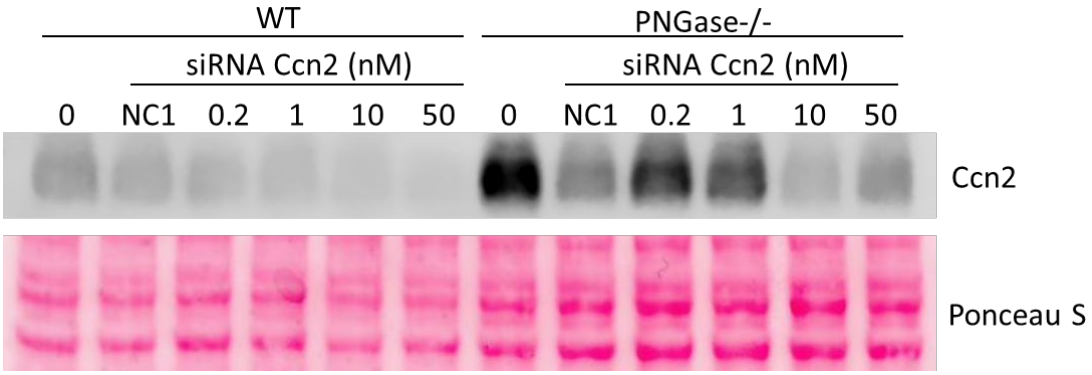

Figure S4. siRNA knock down of Ccn2 in MEFs. WT and PNGase -/- MEFs were transfected with increased amount of Ccn2 siRNA and 50nM scrambled siRNA (NC1) followed by WB analysis of the expression of Ccn2. Ponceau S stain serve as loading control.