Supplementary Information

Materials and methods

Cell viability
Cell viability was assessed using the Cell Counting Kit-8 assay (CCK-8, Beyotime) according to the manufacturer’s instructions. First, $1 \times 10^4$ cells/well were seeded in 96-well plates and exposed to ND for different times. Thereafter, 10 μL of CCK-8 reaction solution was added to each well, cultured at 37 °C for 2 h, and the absorbance value was measured at 450 nm using a spectrophotometer (BioTek, Winooski, VT).

Western blot analysis
The corresponding protein extraction kits (Beyotime, Nantong, China) were used to lyse cells and extract protein samples according to the kit instructions. 40 μg protein of each sample were loaded onto appropriate 8%-12% sodium dodecyl sulfate-polyacrylamide gels to separate protein bands based on the molecular weight of the target protein and transferred to polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany). Then, the membranes were incubated with primary antibodies at the appropriate dilutions overnight at 4 °C prior to incubation with horseradish peroxidase-conjugated secondary antibodies for 2 h at room temperature, detection with an enhanced chemiluminescence imaging system (Bio-Rad) and quantification of band densities with ImageJ software. The antibody dilutions were as follows: anti-OGT (1:1000), anti-O-GlcNAc (1:1000), anti-p16 (1:800), anti-p21 (1:2000), anti-p53
(1:1000), anti-Bcl-2 (1:1000), anti-Bax (1:2000), anti-cleaved caspase 3 (1:1000), anti-
FAM134B (1:1000), anti-LC3B (1:600), anti-p62 (1:1000), anti-ubiquitin (1:1000), and
anti-beta actin (1:2000). The dilutions of the secondary antibodies were as follows: goat
anti-mouse IgG (H+L)-HRP (1:2000), goat anti-rabbit IgG (H+L)-HRP (1:2000), and
mouse anti-rabbit IgG light chain-HRP (1:5000).

**Immunofluorescence staining**

Human NP cells seeded on coverslips were fixed with 4% paraformaldehyde for 20 min
at room temperature, washed three times with PBS, 0.3% Triton X-100 was used to
permeabilize for 15 min. Next, 10% goat serum was applied to block nonspecific
binding for 30 min. The cells were incubated overnight at 4 °C with primary antibodies
against OGT (1:100), O-GlcNAc (1:200), FAM134B (1:100), LC3B (1:100), p16
(1:100), and cleaved caspase 3 (1:100). Then, after washed three times with 0.1% PBST,
the slides were incubated with CoraLite488- or CoraLite594-conjugated goat anti-
rabbit/mouse secondary antibodies (1:100, Proteintech) for 1 h at room temperature.
Fluorescence signals were visualized by fluorescence microscopy (Olympus, BX53,
Melville, NY, USA) and analyzed using ImageJ software.

**Apoptosis assay**

An Annexin V-FITC/PI Apoptosis Detection Kit (KeyGEN, Nanjing, China) was used
to evaluate apoptosis according to the manufacturer’s instructions. In brief, after the
indicated treatment, NP cells were harvested with 0.25% trypsin (containing no EDTA).
After washed three times with PBS, the cells were stained with Annexin V-FITC (annexin V) and propidium iodide (PI) and analyzed using a flow cytometer (BD Biosciences, San Jose, CA, USA). the labeled cells of Annexin V+/PI- and Annexin V+/PI+ were considered as the apoptotic cells.

**Transmission electron microscopy**

Transmission electron microscopy (TEM) was used to detect the formation of autophagosomes and autolysosomes. In brief, the cells were collected and fixed with 2.5% glutaraldehyde for 4 h, rinsed in 0.1 M phosphate buffer (pH 7.4) and subsequently fixed with 1% osmium tetroxide (OsO₄) at room temperature for 2 h. After fixation, the samples were dehydrated twice through an ethanol gradient (30%-50%-70%-80%-85%-90%-95%-100%) and embedded in epoxy resin. After ultrathin (70 nm) sectioning, the sections were double stained with 2% uranyl acetate and lead citrate for 20 min at room temperature and examined using transmission electron microscopy (Tecnai, FEI, USA).

**Coimmunoprecipitation**

Cell samples were lysed with NP-40 lysis buffer (1% NP-40, 30 mM Tris-HCl (pH 7.4), 10 μg/mL aprotinin, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/mL leupeptin) containing protease inhibitor cocktail (MCE) for 20 min on ice, the lysates were then centrifuged at 12,000 × g at 4 °C for 10 min, the supernatants were collected. Then, a bicinchoninic acid (BCA) kit (Beyotime) was used to measure the
total protein concentrations. After preclearing with 20 µL of protein A/G magnetic beads for 1 h at 4 °C, equal amounts of supernatants were incubated with IgG, anti-OGT, and anti-FAM134B antibodies plus protein A/G or anti-HA magnetic beads and anti-Flag magnetic bead conjugates overnight at 4 °C. Then, the beads were washed three times with NP-40 lysis buffer and boiled in 2 × SDS sample loading buffer for subsequent western blot analysis.
Supplemental Figures:

**Supplementary Fig. 1** The protein expression profile of OGA in human NP tissues.

The protein expression level of OGA in human NP tissues was detected using IHC staining, representative images were shown (a) and relative OGA immunopositive staining cell ratio were calculated (b), scale bar: 250 μm and 100 μm. The data are presented as the mean ± S.D. values. *P < 0.05.

**Supplementary Fig. 2** Effect of ND treatment, TMG, and OSMI-1 on cell viability in human NP cells. After exposed to ND treatment (a), TMG (b), and OSMI-1 (c) for the indicated time points (0, 6, 12, 24, and 36 hours), the viability of the human NP cells was measured by CCK-8 assay. The data are presented as the mean ± S.D. values. **P < 0.01, *P < 0.05.**
Supplementary Fig.3 Validation of OGT overexpression and knockdown in human NP cells.

(a, b) 72 hours post lentiviral transfection, the human NP cells were harvested and OGT expression were analyzed using western blot analysis, and the relative band densities were quantified. (c, d) 48 hours post siRNA transfection, the human NP cells were harvested and OGT expression were analyzed using western blot analysis, and the relative band densities were quantified. The data are presented as the mean ± S.D. values. **P < 0.01, *P < 0.05.
Supplementary Fig. 4 Effects of OGA knockdown on ND-induced senescence and apoptosis in human NP cells. (a, b) after transfected with OGA siRNA for 48 hours, the OGA knockdown efficacy in NP cells was validated by western blot analysis, and the relative band densities were quantified. (c-f) NP cells were transduced with the OGA siRNA for 48 h and then subjected to ND treatment for 36 h. The senescence- and apoptosis-associated proteins p53, p21, p16, Bcl-2, Bax, and cleaved caspase 3 were evaluated using western blot analysis, and the relative band densities were quantified. The data are presented as the mean ± S.D. values. **P < 0.01, *P < 0.05.
Supplementary Fig. 5 Effects of OGA knockdown on ND-induced FAM134B-mediated ER-phagy activation in human NP cells. (a, b) NP cells were transduced with the OGA siRNA for 48 h and then subjected to ND treatment for 36 h. ER-phagy associated proteins FAM134B, LC3, and p62 were assessed with western blot analysis, and the relative band densities were quantified. The data are presented as the mean ± S.D. values. **P < 0.01, *P < 0.05.

Supplementary Fig. 6 Validation of FAM134B knockdown in human NP cells. (a, b) 48 hours post siRNA transfection, the human NP cells were harvested and FAM134B expression were analyzed using western blot analysis, and the relative band densities were quantified. The data are presented as the mean ± S.D. values. **P < 0.01, *P < 0.05.
### Supplementary Tables

#### Supplementary Table 1 Patient demographics

| Case number | Gender | Age (years) | Pfirrmann grade | Diagnosis                        |
|-------------|--------|-------------|-----------------|----------------------------------|
| Case 1      | male   | 13          | I               | Adolescent idiopathic scoliosis  |
| Case 2      | female | 11          | I               | Adolescent idiopathic scoliosis  |
| Case 3      | female | 14          | II              | Adolescent idiopathic scoliosis  |
| Case 4      | male   | 17          | II              | Adolescent idiopathic scoliosis  |
| Case 5      | female | 17          | II              | Adolescent idiopathic scoliosis  |
| Case 6      | male   | 21          | II              | Adolescent idiopathic scoliosis  |
| Case 7      | female | 43          | IV              | Lumbar spinal stenosis           |
| Case 8      | female | 52          | IV              | Lumbar spinal stenosis           |
| Case 9      | male   | 57          | IV              | Lumbar spinal stenosis           |
| Case 10     | female | 61          | V               | Lumbar spinal stenosis           |
| Case 11     | male   | 63          | V               | Lumbar spinal stenosis           |
| Case 12     | male   | 58          | IV              | Lumbar spinal stenosis           |
### Supplementary Table 2 Reagents and antibodies used in this study

| Product name                      | Manufacturer      | Catalog No. |
|-----------------------------------|-------------------|-------------|
| DMEM/F12                          | Gibco, USA        | 11330032    |
| FBS                               | Gibco, USA        | 16000-044   |
| PBS                               | Gibco, USA        | 70011-044   |
| glucose-free DMEM                 | Gibco, USA        | 11966-025   |
| thiamet G                         | MedChemExpress, USA | HY-12588   |
| protein A/G magnetic beads        | MedChemExpress, USA | HY-K0202   |
| anti-HA magnetic beads            | MedChemExpress, USA | HY-K0201   |
| anti-Flag magnetic beads          | MedChemExpress, USA | HY-K0207   |
| OSMI-1                            | Abcam, USA        | ab235455    |
| Cycloheximide                     | Selleck, USA      | S7418       |
| ER-Tracker Green                  | Yeasen, China     | 40763ES20   |
| LysoTracker Red                   | Yeasen, China     | 40739ES50   |
| anti-OGT                          | Abcam, USA        | ab96718     |
| anti-p21                          | Abcam, USA        | ab109520    |
| anti-Bcl-2                        | Abcam, USA        | ab196495    |
| anti-Bax                          | Abcam, USA        | ab104156    |
| anti-LC3B                         | CST, USA          | # 83506     |
| anti-p16                          | CST, USA          | #18769      |
| anti-cleaved caspase 3            | CST, USA          | #9664       |
| anti-FAM134B                      | Proteintech, China | 21537-1-AP |


| Antibody                        | Supplier        | Code       |
|--------------------------------|-----------------|------------|
| anti-p62                       | Proteintech, China | 18420-1-AP |
| anti-p53                       | Proteintech, China | 10442-1-AP |
| anti-ubiquitin                 | Proteintech, China | 10201-2-AP |
| anti-beta actin                | Proteintech, China | 66009-1-Ig |
| anti-O-GlcNAc                  | Novus, USA       | 10442-1-AP |
| goat anti-mouse IgG (H+L)-HRP | Abcam, USA       | ab6789     |
| goat anti-rabbit IgG (H+L)-HRP| Abcam, USA       | ab6721     |
| mouse anti-rabbit IgG chain-HRP| Abcam, USA       | ab99697    |
| goat anti-rabbit CoraLite488   | Proteintech, China | SA00013-2 |
| goat anti-mouse CoraLite594    | Proteintech, China | SA00013-3 |
| goat anti-mouse CoraLite488    | Proteintech, China | SA00013-1 |
| goat anti-rabbit CoraLite594   | Proteintech, China | SA00013-4 |