Supplementary Data

1. EXPERIMENTAL PROCEDURES

1.1 Animals

The study was performed using 4 weeks old C57BL/6J mice, 4 weeks old Sprague-Dawley (SD) rats, and 3 months old pigs. Mice were housed in standard cages in a specific pathogen-free facility of Sun Yat-sen University. The room was maintained on a 12h light-dark cycle at a constant temperature of 25°C and 50% humidity and the animals were fed with commercial laboratory food and sterilized water. SD rats were purchased from Zhongshan Medical School of Sun Yat-sen University. The 3 months old pigs were raised in the animal facility of Hunan Normal University. In all the cases, animal protocols to use the above species were approved by the IACUC of Zhongshan Ophthalmic Center of Sun Yat-sen University.

1.2 Collection of lens capsular epithelial samples

Collection of human capsular epithelia from cataract lenses of different age groups was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center (ZOC). Informed consent was obtained from each of the cataract patients. For senile cataractous samples, the lens capsules from cataract patients were collected at surgery by the physicians in Zhongshan Ophthalmic Center of Sun Yat-sen University. According to the patient age, capsular samples from 50 to 59 years old were pooled together and labeled as 50s (Table S1); those from 60 to 69 years old were pooled together and labeled as 60s (Table S2); those from 70 to 79 years old were pooled together and labeled as 70s (Table S3), and those from 80 to 89 years old were pooled together and labeled as 80s
(Table S4). For complicated cataractous samples, patients with cataract and other complications including diabetes, glaucoma or other syndromes (Table S5) were pooled together for the study described in figure 5. As control, the capsular samples from the lenses of human donors (two female individuals with age of 45 and 56, and one male individual with age of 65), adult mice, rats and pigs as described in the above section were dissected out in the laboratory.

1.3 Lens organ culture

The 4 weeks old C57BL/6J mice were sacrificed by CO₂ inhalation. The eyeballs were removed and the lenses were carefully dissected by a posterior approach (Li et al., 1995, Li and Spector, 1996). Dissected lenses were placed in a 10-cm dish containing 20 ml medium199, then incubated at 37°C with a 5% CO₂ gas phase for 12h. The medium 199 was prepared with ion-exchange double-distilled water and supplemented with 26mM NaHCO₃, with a pH adjusted to 7.2, then sterilized by filtration through 0.22um filter. After cultured for 12h, transparent lenses were selected for further experimentation.

1.4 Glucose oxidase (GO) treatment

For each sample, three transparent lenses were transferred into a 6-cm petri dish containing 7 ml of medium 199 supplemented with 10 mU glucose oxidase (GO) (Li et al., 1995; Gong et al., 2018), which continuously generates cytotoxic H₂O₂ in an average of 100 μM in a 24 h period.
1.5 Apoptosis assays

The percentage of apoptosis in GO-treated mouse lenses were determined by cellTiter-Glo® luminescent cell viability assay kit (G7573, Promega) (Crouch et al., 1993) and verified with live/dead viability/cytotoxicity kit (L3224, Thermofisher Scientific) according to the company instruction.

1.6 Total protein extraction and western blot analysis

Total proteins were extracted from cultured animal lenses with RIPA buffer (50 mM Tris·HCl (pH7.4), 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS, 1% sodium deoxyholate) and homogenized as described below (Li et al., 1995; Li and Spector, 1996; Li et al, 2005; Yan et al., 200 and 2010; Gong et al., 2014 and 2018). For various capsular samples of lens epithelium, each pooled capsular samples from 23 to 41 patients (see Table S1 to S5 for details), or from 20 animal lenses were transferred to an Eppendorf tube containing 200 µl RIPA buffer and homogenized on ice with an Eppendorf tube micropostle (Brinkmann Instruments Inc.). For each sample, the protein concentration was determined as previously described (Li et al., 2006; Xiao et al., 2010). 100 µg of total proteins in each sample were separated by 8% SDS-PAGE gel and transferred into PVDF membranes. The protein blots were blocked with 5% non-fat milk in TBST (10 mM Tris HCl/pH8.0, 150 mM NaCl, 0.05% Tween-20) for 1 h at room temperature. Each membrane was then incubated with anti-Pax6 (rabbit monoclonal, 1:1000, CST, SAB4501345), anti-SUMO1 (rabbit polyclonal, 1:1000, CST, SAB1300037), SUMO2/3 (1:500; Enzo Life Sciences), anti-AOS1 (mouse monoclonal, 1:100, Santa Curz Biotechnology, sc376628), anti-UBA2 (rabbit polyclonal, 1:1000,
CST, 5293), anti-Ubc9 (rabbit monoclonal, 1:1000, CST, 4786), anti-PIAS1 (rabbit monoclonal, 1:1000, CST, 3550), anti-RanBP2 (mouse monoclonal, 1:500, Santa Curz Biotechnology, sc74518), anti-SENP1 (rabbit monoclonal, 1:1000, Sigma, SAB4501345), anti-SENP2 (rabbit polyclonal, 1:1000, Sigma, SAB1300037), anti-SENP3 (rabbit monoclonal, 1:1000, CST, 5591), anti-SENP5 (rabbit polyclonal, 1:1000, Proteintech, 19529-1-AP), anti-SENP6 (mouse monoclonal, 1:500, Santa Curz Biotechnology, sc-100585), anti-SENP7 (mouse monoclonal, 1:500, Santa Curz Biotechnology, sc-373821), anti-SENP8 (mouse monoclonal, 1:500, Santa Curz Biotechnology, sc-271498) and anti-β-Actin (mouse monoclonal, 1:1000, Beijing Ray Antibody Biotech, RM2001) at 4°C overnight with mild shaking. After three 10 min washes with TBST, each blot was incubated with the HRP-conjugated secondary antibody (anti-mouse and rabbit IgG from CST) diluted at 1:2000 in blocking solution at room temperature for 1 h. The blots were visualized using a Tanon chemiluminescence system (China).

1.7 Automated western immunoblotting

The simple western immunoblots were performed on a PeggySue (ProteinSimple) as previously described (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 µg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using the antibodies described above. The dilution factors are 1:100 for Pax6, Ubc9, Uba2, PIAS1, SENP3, SUMO2/3, and 1:50 for SENP1, SENP2, and SUMO1, as well as 1:20 for AOS1, RanBP2, SENP6, SENP7 and SENP8. The Campass software (Protein Simple, version
4.1.5) was used to program the PeggSue-robot and for presentation (and quantification) of the western blots. Output western blot style data were displayed with exposure time indicated, and the quantification data were displayed from the software-calculated average of seven exposures (1-512 s).

1.8 Protein solubility analysis

To compare protein solubility of human capsular epithelium samples from different age groups, total protein from each sample was first extracted as described above. The supernatant was considered as soluble protein. The remaining pellet was dissolved in modified protein extraction buffer with 5% CHAPs to replace 1% NP-40, and the suspended protein solutions were further sonicated on ice for 3 min, immersed in ice for 3 min. This cycle was repeated 5 times to help dissolving the pellet, the protein solution was then used for determination of protein concentration as described (Marshak et al. 1998). The protein solubility (Table S6) was calculated by dividing the initial soluble proteins with the total proteins from combination of two extractions.

1.9 References for Experimental Procedures

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## 2. SUPPLEMENTARY TABLES

Table S1. Prenatal cataract patients’ information (50-59 years old)

| Number | Gender | Age | Diagnosed subtype       |
|--------|--------|-----|-------------------------|
| P1     | Female | 50  | Nuclear Cataract        |
| P2     | Female | 50  | Cortical Cataract       |
| P3     | Female | 51  | Cortical Cataract       |
| P4     | Female | 51  | Nuclear Cataract        |
| P5     | Female | 51  | Cortical Cataract       |
| P6     | Female | 51  | Cortical Cataract       |
| P7     | Male   | 51  | Cortical Cataract       |
| P8     | Male   | 52  | Cortical Cataract       |
| P9     | Male   | 51  | Cortical Cataract       |
| P10    | Male   | 52  | Nuclear Cataract        |
| P11    | Male   | 53  | Cortical Cataract       |
| P12    | Female | 53  | Nuclear Cataract        |
| P13    | Female | 55  | Nuclear Cataract        |
| P14    | Female | 55  | Cortical Cataract       |
| P15    | Female | 56  | Cortical Cataract       |
| P16    | Female | 56  | Cortical Cataract       |
| P17    | Female | 56  | Cortical Cataract       |
| P18    | Male   | 56  | Nuclear Cataract        |
| P19    | Male   | 57  | Nuclear Cataract        |
| P20    | Female | 57  | Nuclear Cataract        |
| P21    | Female | 57  | Cortical Cataract       |
| P22    | Female | 57  | Cortical Cataract       |
| P23    | Male   | 58  | Cortical Cataract       |
| P24    | Female | 58  | Nuclear Cataract        |
| P25    | Male   | 58  | Cortical Cataract       |
| P26    | Female | 59  | Cortical Cataract       |
| P27    | Female | 59  | Cortical Cataract       |
| P28    | Female | 57  | Nuclear Cataract        |
| P29    | Female | 58  | Cortical Cataract       |
| P30    | Male   | 58  | Nuclear Cataract        |
| P31    | Male   | 59  | Nuclear Cataract        |

Samples displayed in red were used for automated western blot analysis.
Table S2. Senile cataract patients’ information (60-69 years old)

| Number | Gender | Age | Diagnosed subtype                  |
|--------|--------|-----|-----------------------------------|
| P1     | Female | 60  | Cortical Cataract                 |
| P2     | Female | 60  | Cortical Cataract                 |
| P3     | Male   | 60  | Nuclear Cataract                  |
| P4     | Female | 61  | Cortical Cataract                 |
| P5     | Female | 62  | Nuclear Cataract                  |
| P6     | Male   | 62  | Cortical Cataract                 |
| P7     | Male   | 62  | Cortical Cataract                 |
| P8     | Female | 63  | Nuclear Cataract                  |
| P9     | Female | 63  | Cortical Cataract                 |
| P10    | Female | 63  | Cortical Cataract                 |
| P11    | Female | 63  | Cortical Cataract                 |
| P12    | Male   | 63  | Nuclear Cataract                  |
| P13    | Female | 64  | Cortical Cataract                 |
| P14    | Male   | 64  | Nuclear Cataract                  |
| P15    | Male   | 65  | Nuclear Cataract                  |
| P16    | Female | 65  | Cortical Cataract                 |
| P17    | Female | 65  | Cortical Cataract                 |
| P18    | Female | 65  | Nuclear Cataract                  |
| P19    | Female | 65  | Cortical Cataract                 |
| P20    | Male   | 65  | Nuclear Cataract                  |
| P21    | Female | 66  | Cortical Cataract                 |
| P22    | Male   | 66  | Cortical Cataract                 |
| P23    | Female | 66  | Cortical Cataract                 |
| P24    | Male   | 66  | Cortical Cataract                 |
| P25    | Female | 66  | Nuclear Cataract                  |
| P26    | Female | 66  | Cortical Cataract                 |
| P27    | Female | 66  | Cortical Cataract                 |
| P28    | Female | 67  | Cortical Cataract                 |
| P29    | Female | 67  | Nuclear Cataract                  |
| P30    | Female | 67  | Nuclear Cataract                  |
| P31    | Female | 67  | Cortical Cataract                 |
| P32    | Female | 68  | Cortical Cataract                 |
| P33    | Male   | 68  | Nuclear Cataract                  |
| P34    | Male   | 69  | Nuclear Cataract                  |
| P35    | Female | 67  | Nuclear Cataract                  |
| P36    | Female | 67  | Nuclear Cataract                  |
| P37    | Male   | 67  | Posterior Subcapsular Cataract    |
| P38    | Male   | 68  | Nuclear Cataract                  |

Samples displayed in red were used for automated western blot analysis.
Table S3. Senile cataract patients’ information (70-79 years old)

| Number | Gender | Age | Diagnosed subtype       |
|--------|--------|-----|-------------------------|
| P1     | Female | 70  | Cortical Cataract       |
| P2     | Female | 70  | Cortical Cataract       |
| P3     | Female | 71  | Cortical Cataract       |
| P4     | Male   | 71  | Nuclear Cataract        |
| P5     | Male   | 71  | Nuclear Cataract        |
| P6     | Female | 72  | Cortical Cataract       |
| P7     | Female | 72  | Cortical Cataract       |
| P8     | Male   | 72  | Cortical Cataract       |
| P9     | Female | 73  | Cortical Cataract       |
| P10    | Male   | 73  | Cortical Cataract       |
| P11    | Female | 73  | Cortical Cataract       |
| P12    | Female | 73  | Nuclear Cataract        |
| P13    | Male   | 73  | Cortical Cataract       |
| P14    | Female | 73  | Cortical Cataract       |
| P15    | Male   | 74  | Nuclear Cataract        |
| P16    | Female | 74  | Nuclear Cataract        |
| P17    | Male   | 74  | Nuclear Cataract        |
| P18    | Male   | 74  | Cortical Cataract       |
| P19    | Female | 75  | Nuclear Cataract        |
| P20    | Female | 75  | Cortical Cataract       |
| P21    | Female | 75  | Nuclear Cataract        |
| P22    | Male   | 75  | Nuclear Cataract        |
| P23    | Female | 76  | Cortical Cataract       |
| P24    | Male   | 76  | Nuclear Cataract        |
| P25    | Male   | 76  | Nuclear Cataract        |
| P26    | Female | 76  | Cortical Cataract       |
| P27    | Female | 77  | Cortical Cataract       |
| P28    | Female | 77  | Nuclear Cataract        |
| P29    | Female | 78  | Nuclear Cataract        |
| P30    | Female | 78  | Cortical Cataract       |
| P31    | Male   | 78  | Cortical Cataract       |
| P32    | Female | 79  | Cortical Cataract       |
| P33    | Male   | 79  | Cortical Cataract       |
| P34    | Male   | 75  | Nuclear Cataract        |
| P35    | Male   | 79  | Nuclear Cataract        |
| P36    | Female | 79  | Nuclear Cataract        |
| P37    | Female | 79  | Nuclear Cataract        |

Samples displayed in red were used for automated western blot analysis.
Table S4. Senile cataract patients’ information (80-90 years old)

| Number | Gender | Age | Diagnosed subtype       |
|--------|--------|-----|-------------------------|
| P1     | Male   | 80  | Nuclear Cataract        |
| P2     | Female | 80  | Nuclear Cataract        |
| P3     | Female | 81  | Cortical Cataract       |
| P4     | Male   | 81  | Nuclear Cataract        |
| P5     | Male   | 81  | Cortical Cataract       |
| P6     | Male   | 81  | Nuclear Cataract        |
| P7     | Female | 82  | Cortical Cataract       |
| P8     | Female | 82  | Nuclear Cataract        |
| P9     | Male   | 82  | Nuclear Cataract        |
| P10    | Female | 83  | Cortical Cataract       |
| P11    | Male   | 83  | Cortical Cataract       |
| P12    | Male   | 84  | Cortical Cataract       |
| P13    | Female | 84  | Cortical Cataract       |
| P14    | Female | 86  | Cortical Cataract       |
| P15    | Female | 86  | Cortical Cataract       |
| P16    | Male   | 87  | Cortical Cataract       |
| P17    | Male   | 88  | Nuclear Cataract        |
| P18    | Female | 88  | Cortical Cataract       |
| P19    | Female | 89  | Nuclear Cataract        |
| P20    | Female | 89  | Nuclear Cataract        |
| P21    | Female | 89  | Cortical Cataract       |
| P22    | Female | 89  | Nuclear Cataract        |
| P23    | Male   | 90  | Cortical Cataract       |
| P24    | Male   | 83  | Nuclear Cataract        |
| P25    | Male   | 85  | Nuclear Cataract        |
| P26    | Female | 83  | Cortical Cataract       |
| P27    | Female | 84  | Nuclear Cataract        |

Samples displayed in red were used for automated western blot analysis.
Table S5. Complicated cataract patients’ information

| Number | Gender | Age | Complication          |
|--------|--------|-----|-----------------------|
| P1     | Male   | 39  | High Myopia           |
| P2     | Female | 48  | Diabetes              |
| P3     | Female | 50  | Glaucoma              |
| P4     | Male   | 51  | High Myopia           |
| P5     | Female | 52  | High Myopia           |
| P6     | Female | 54  | Glaucoma              |
| P7     | Female | 55  | High Myopia           |
| P8     | Male   | 56  | Retinal Detachment    |
| P9     | Female | 57  | Glaucoma              |
| P10    | Female | 57  | Diabetes              |
| P11    | Male   | 59  | Diabetes              |
| P12    | Female | 60  | Retinal Detachment    |
| P13    | Male   | 61  | Uveitis               |
| P14    | Female | 61  | Retinal Detachment    |
| P15    | Female | 61  | Retinitis Pigmentosa  |
| P16    | Male   | 63  | Glaucoma              |
| P17    | Male   | 63  | Uveitis               |
| P18    | Male   | 64  | Retinitis Pigmentosa  |
| P19    | Female | 64  | Retinitis Pigmentosa  |
| P20    | Female | 66  | Diabetes              |
| P21    | Male   | 67  | Glaucoma              |
| P22    | Male   | 70  | Retinal Detachment    |
| P23    | Female | 71  | Glaucoma              |
| P24    | Male   | 72  | Retinal Detachment    |
| P25    | Male   | 72  | Glaucoma              |
| P26    | Female | 72  | Retinal Detachment    |
| P27    | Male   | 74  | High Myopia           |
| P28    | Male   | 75  | Uveitis               |
| P29    | Female | 76  | Diabetes              |
| P30    | Female | 77  | Diabetes              |
| P31    | Female | 78  | Retinitis Pigmentosa  |
| P32    | Female | 79  | Retinal Detachment    |
| P33    | Male   | 79  | Glaucoma              |
| P34    | Female | 79  | Glaucoma              |
| P35    | Female | 80  | Diabetes              |
| P36    | Male   | 80  | Glaucoma              |
| P37    | Male   | 81  | Retinitis Pigmentosa  |
| P38    | Male   | 81  | Glaucoma              |
| P39    | Male   | 85  | Glaucoma              |
| P40    | Male   | 89  | Glaucoma              |
| P41    | Male   | 90  | Retinal Detachment    |
| P42    | Female | 23  | Uveitis               |
|    | Gender | Age | Diagnosis                  |
|----|--------|-----|----------------------------|
| P43| Female | 29  | Retinal Detachment         |
| P44| Male   | 34  | Retinitis Pigmentosa       |
| P44| Male   | 38  | High Myopia                |
| P45| Male   | 38  | Glaucoma                   |
| P46| Male   | 43  | High Myopia                |
| P47| Male   | 44  | Hypertension               |
| P48| Female | 45  | Retinal Detachment         |
| P49| Male   | 47  | Uveitis                    |
| P50| Male   | 48  | Glaucoma                   |
| P51| Female | 49  | High Myopia                |
| P52| Male   | 50  | Retinitis Pigmentosa       |
| P53| Female | 53  | Retinal Detachment         |
| P54| Female | 53  | Diabetes                   |
| P55| Male   | 62  | Hypertension               |
| P56| Male   | 63  | Retinal Detachment         |
| P57| Female | 64  | Diabetes                   |

Samples displayed in red were used for automated western blot analysis.
Table S6. Result of the soluble proteins in different age group samples

| Age groups | Total protein (µg) | Soluble protein (µg) | Insoluble protein (µg) | The proportion of soluble protein |
|------------|--------------------|----------------------|------------------------|---------------------------------|
| 50s        | 287.12             | 254.17               | 32.95                  | 100%                            |
| 60s        | 215.73             | 188.27               | 27.46                  | 87%                             |
| 70s        | 207.89             | 180.43               | 27.46                  | 87%                             |
| 80s        | 211.02             | 178.08               | 32.94                  | 84%                             |
3. LEGENDS TO SUPPLEMENTARY FIGURES

FIGURE S1. Automated western blot analysis of ligase 1 subunits, AOS1 and UBA2 in normal and cataractous human lenses of different age groups. The automated western immunoblots were performed on a PeggySue (ProteinSimple) as described recently (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 μg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using anti-AOS1 and anti-UBA2 antibodies (for antibody information, see Experimental Procedures) with a dilution factor of 1:20 and 1:100, respectively. The Campass software (Protein Simple, version 4.1.5) was used to program the PeggySue-robot and for presentation (A) and quantification (B-E). Output western blot style data (A) were displayed with exposure time indicated, and the quantification data (B-E) were displayed from the software-calculated average of seven exposures (1-512 s). A. Besides AOS1 and UBA2 samples, β-actin with 1 second exposure was included as loading control. B and C. Quantification results show gender difference. Each bar represents an average of two samples for cataract lenses but one sample for normal human lens. D and E. Quantification results show age difference.

FIGURE S2. Automated western blot analysis of ligase 2, UBC9 in normal and cataractous human lenses of different age groups. The automated western immunoblots were performed on a PeggySue (ProteinSimple) as described recently (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 μg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using anti-UBC9 antibody (for antibody information,
see Experimental Procedures) with a dilution factor of 1:100. The Campass software (Protein Simple, version 4.1.5) was used to program the PeggSue-robot and for presentation (A) and quantification (B-C). Output western blot style data (A) were displayed with exposure time indicated, and the quantification data (B-C) were displayed from the software-calculated average of seven exposures (1-512 s). B. Quantification results show gender difference. Each bar represents an average of two samples for cataract lenses but one sample for normal human lens. C. Quantification results show age difference.

**FIGURE S3. Automated western blot analysis of ligase 3, PIAS1 in normal and cataractous human lenses of different age groups.** The automated western immunoblots were performed on a PeggySue (ProteinSimple) as described recently (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 μg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using anti-PIAS1 antibody (for antibody information, see Experimental Procedures) with a dilution factor of 1:100. The Campass software (Protein Simple, version 4.1.5) was used to program the PeggSue-robot and for presentation (A) and quantification (B-C). Output western blot style data (A) were displayed with exposure time indicated, and the quantification data (B-C) were displayed from the software-calculated average of seven exposures (1-512 s). B. Quantification results show gender difference. Each bar represents an average of two samples for cataract lenses but one sample for normal human lens. C. Quantification results show age difference.
FIGURE S4. Automated western blot analysis of desumoylation enzyme, SENP3 in normal and cataractous human lenses of different age groups. The automated western immunoblots were performed on a PeggySue (ProteinSimple) as described recently (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 μg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using anti-SENP3 antibody (for antibody information, see Experimental Procedures) with a dilution factor of 1:100. The Campass software (Protein Simple, version 4.1.5) was used to program the PeggySue-robot and for presentation (A) and quantification (B-C). Output western blot style data (A) were displayed with exposure time indicated, and the quantification data (B-C) were displayed from the software-calculated average of seven exposures (1-512 s). B. Quantification results show gender difference. Each bar represents an average of two samples for cataract lenses but one sample for normal human lens. C. Quantification results show age difference.

FIGURE S5. Automated western blot analysis of desumoylation enzyme, SENP7 in normal and cataractous human lenses of different age groups. The automated western immunoblots were performed on a PeggySue (ProteinSimple) as described recently (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 μg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using anti-SENP7 antibody (for antibody information, see Experimental Procedures) with a dilution factor of 1:20. The
Campass software (Protein Simple, version 4.1.5) was used to program the PeggySue-robot and for presentation (A) and quantification (B-C). Output western blot style data (A) were displayed with exposure time indicated, and the quantification data (B-C) were displayed from the software-calculated average of seven exposures (1-512 s). B. Quantification results show gender difference. Each bar represents an average of two samples for cataract lenses but one sample for normal human lens. C. Quantification results show age difference.

FIGURE S6. **Automated western blot analysis of desumoylation enzyme, SENP8 in normal and cataractous human lenses of different age groups.** The automated western immunoblots were performed on a PeggySue (ProteinSimple) as described recently (Dahl et al. 2016). Briefly, each sample was loaded with 0.9 μg total protein and then analyzed with the Size Separation Master Kit and Split Buffer (12-230 kDa) according to the manufacturer’s standard instruction using anti-SENP8 antibody (for antibody information, see Experimental Procedures) with a dilution factor of 1:20. The Campass software (Protein Simple, version 4.1.5) was used to program the PeggySue-robot and for presentation (A) and quantification (B-C). Output western blot style data (A) were displayed with exposure time indicated, and the quantification data (B-C) were displayed from the software-calculated average of seven exposures (1-512 s). B. Quantification results show gender difference. Each bar represents an average of two samples for cataract lenses but one sample for normal human lens. C. Quantification results show age difference.
AOS1: 128 s; UBA2: 512 s; β-Actin: 1 s

Quantification of Aos 1 Amount in 0.9μg Protein (x10^3 Units)

- F: 40s, 50s, 60s
- M: 50s, 60s, 70s, 80s

Normal Lens vs. Cataractous Lens

Quantification of Uba 2 Amount in 0.9μg Protein (x10^3 Units)

- F: 40s, 50s, 60s
- M: 50s, 60s, 70s, 80s

Normal Lens vs. Cataractous Lens

E, F: Normal Lens vs. Cataract Lens

Quantitative Analysis of AOS1 and UBA2
Fig. S2

A

18 kd

UBC9

128 s

B

Quantification of UBC9 Amount in 0.9 μg Protein (x10^3 Units)

| Time  | Normal Lens | Cataractous Lens |
|-------|-------------|------------------|
| 40s   | F           | F                |
| 50s   | F           | M                |
| 60s   | M           | F                |
| 50s   | F           | M                |
| 60s   | M           | F                |
| 70s   | F           | M                |
| 80s   | M           | F                |
| 80s   | F           | F                |

C

Quantification of UBC9 Amount in 0.9 μg Protein (x10^3 Units)

| Time  | Normal Lens | Cataractous Lens |
|-------|-------------|------------------|
| 50s   | *           | ***              |
| 60s   | ***         |                  |
| 70s   | 20          |                  |
| 80s   | 20          |                  |
Fig. S6

A

24 kd

SENp8

4 s

B

Quantification of SENP8 Amount in 0.9 μg Protein (x10^4 Units)

|       | F | F | M | M | F | M | F | M | F | M | F |
|-------|---|---|---|---|---|---|---|---|---|---|---|
| 40s   | 8 | 6 | 6 | 6 | 8 | 6 | 6 | 6 | 8 | 6 | 6 |
| 50s   | 12| 10| 10| 10| 12| 10| 10| 10| 12| 10| 10|
| 60s   | 12| 10| 10| 10| 12| 10| 10| 10| 12| 10| 10|

Normal Lens  Cataractous Lens

C

Quantification of SENP8 Amount in 0.9 μg Protein (x10^4 Units)

|       | Normal Lens | Cataractous Lens |
|-------|-------------|------------------|
| 50s   | ***         | ns               |
| 60s   |             |                  |
| 70s   |             |                  |
| 80s   |             |                  |