

Supplemental Appendix 1: Method for serum heat shock protein (HSP) antibody level measurements

To evaluate the serum levels of anti-HSPs antibodies, medium-bind 96-well plates (Greiner Bio-One) were pre-coated with commercially available full-length recombinant human α crystallin (Enzo Life Sciences), HSP27 (Enzo Life Sciences), or HSP60 (Enzo Life Sciences) proteins at a concentration of 1 μ g/ml and incubated in ELISA Coating Buffer (BioLegend) containing 10% normal goat serum (Sigma-Aldrich) at room temperature for 2 hours. Sera diluted (1:10) in 1xPBS containing 1% BSA were added to the well. Anti- α crystallin (Thermo Scientific), anti-HSP27(Thermo Scientific) or anti-HSP60 antibody (Thermo Scientific) was used as a positive control that was added in replacement of diluted sera, and incubated for 2 hours at room temperature. Plates were then incubated with horseradish peroxidase (HRP)-conjugated anti-human IgG (1:1,000, Novus Biologicals) at room temperature for 30 minutes. The TMB substrate solution (R&D system) was added to visualize HRP enzymatic reaction, and optical density was evaluated at 450 nm using an automatic microplate reader (SYNERGY H1 and Gen 5TM Imager Software, BioTek.). All samples were performed in duplicate.