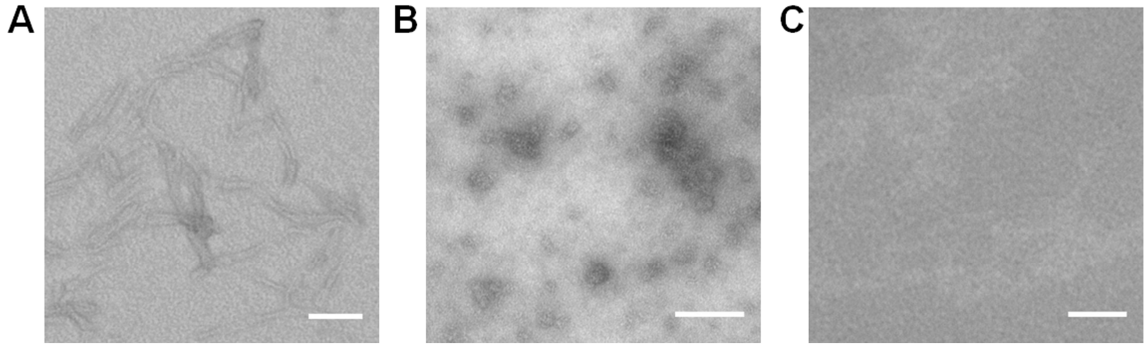
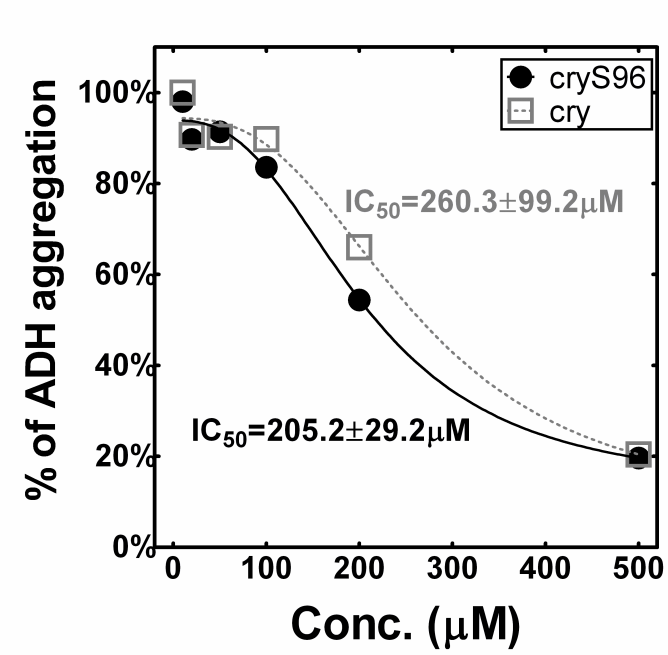


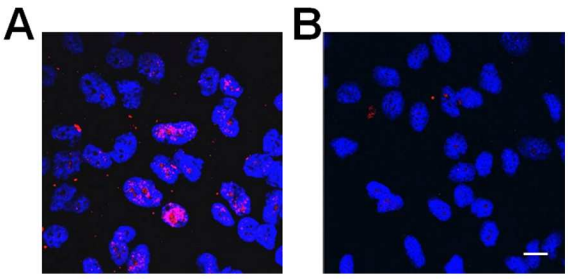
Supplementary Figure S1. Fusion to the S96 protein polymer inhibits the fibril formation for the αB crystallin mini-peptide. Negative stained transmission electron microscopy was used to evaluate the oligomerization and fibril formation of the cry peptide. **A**) The mini-peptide cry alone assembles amyloid-like fibrils. **B**) The scrambled version of the mini-peptide (Neg) formed irregular clusters, but does not form fibrils. **C**) No well-defined oligomers or fibrils were observed for cryS96 alone. Scale bar: 100nm.



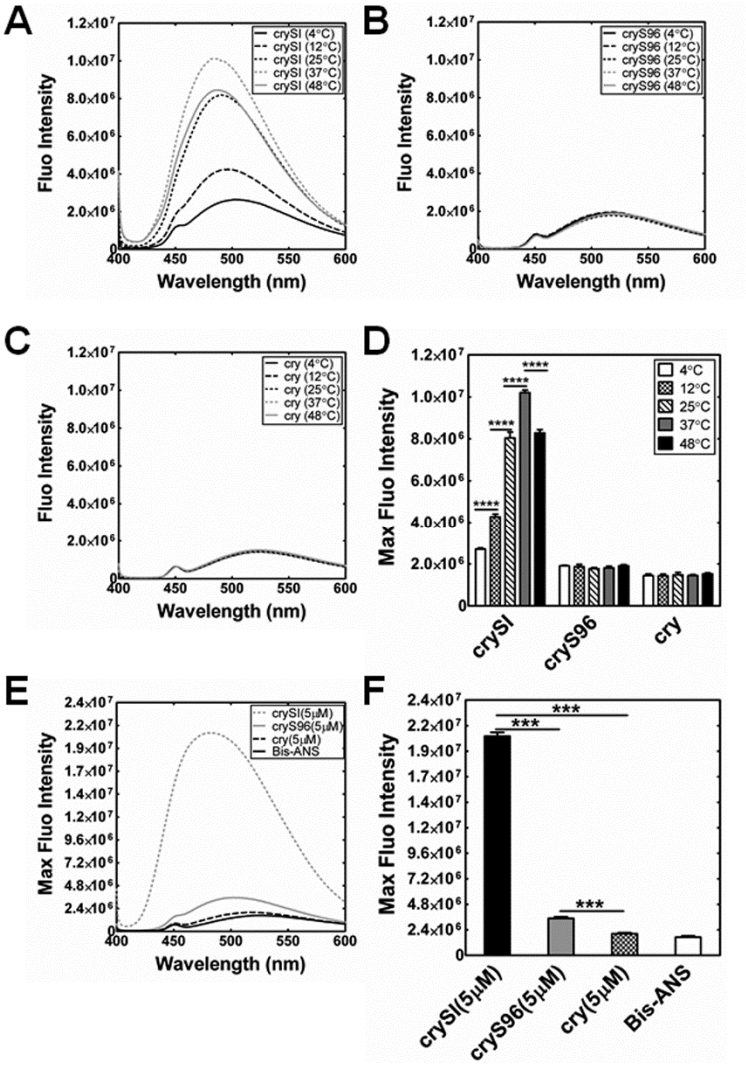
Supplementary Figure S2. cryS96 exhibits concentration dependent chaperone activity against Alcohol Dehydrogenase (ADH). Optical density was used to evaluate the aggregation of ADH (100 µg) at 360 nm after incubation at 48 °C for 60 min vs. ADH alone (100%). Inhibition of ADH aggregation for both cryS96 and cry was dose dependent. Raw data points were independently fit to a sigmoidal curve:

$$\%\ of\ ADH\ aggregation = Bottom + (Top - Bottom) \times [Conc]^H / \left(IC_{50}^H + [Conc]^H\right)$$

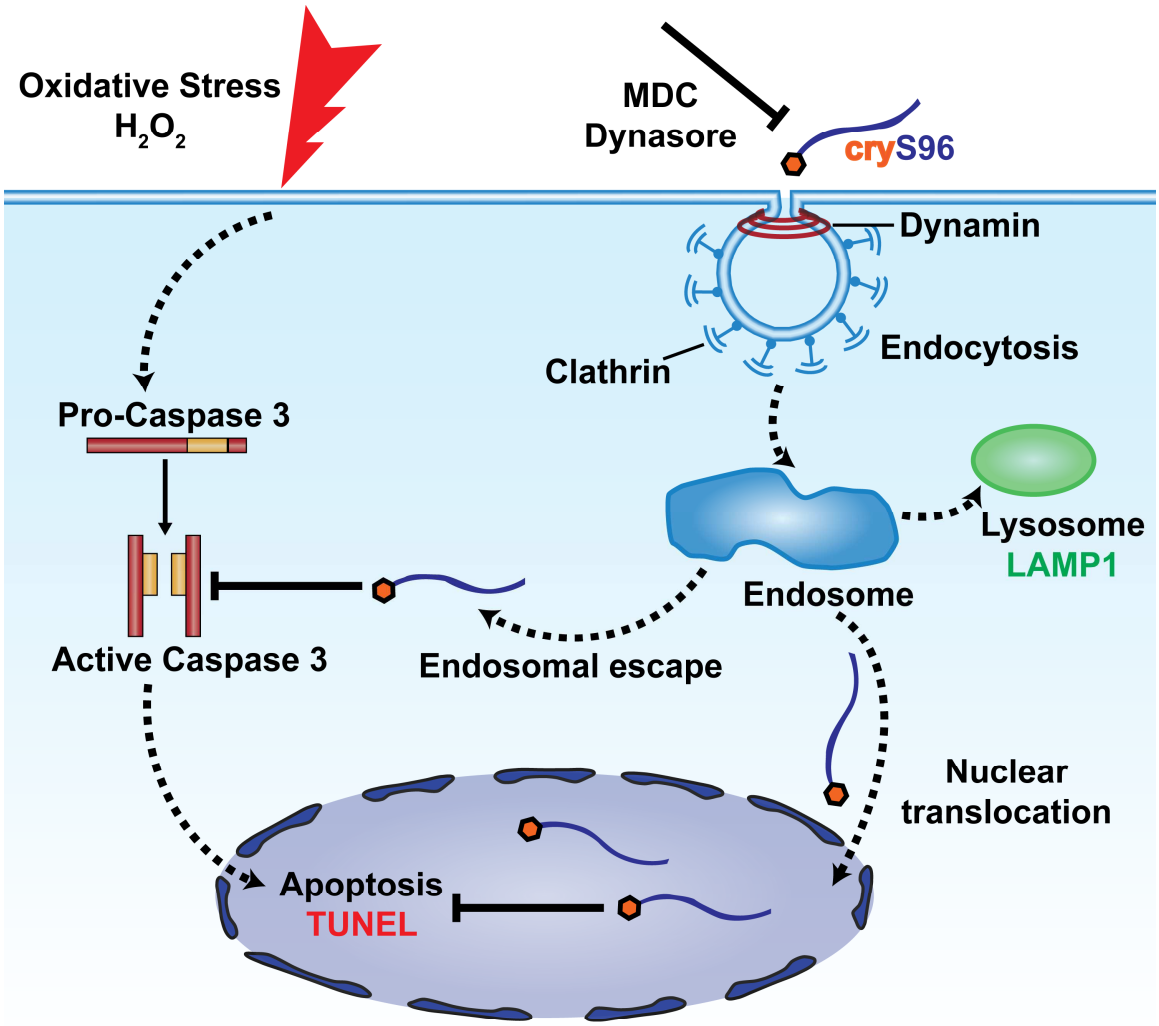
This predicted IC_{50} values are displayed (mean ± SEM) for cry (top) and cryS96 (bottom). $R^2_{crysS96} = 0.9926$; $R^2_{cry} = 0.9871$.



Supplementary Figure S3. Nuclear translocation of cryS96 is dynamin dependent. Human RPE cells were incubated with rhodamine labeled cryS96 (red) and H_2O_2 (200 µM) for 24 hrs in the **A**) absence and **B**) presence of 50 µM dynasore. Nuclei were labeled with DAPI (blue). Scale bar: 20 µm.



Supplementary Figure S4. A fluorescent probe mixed with CrySI reveals a temperature-dependent increase in hydrophobicity. The Bis-ANS (5 μM) probe was used to explore shifts in fusion peptide hydrophobicity for the cry mini-peptide and its ELP fusion proteins, crySI and crys96. Ex: 390nm; Em: 400-600nm; slit: 5nm. **A-D**) Peptides were incubated at 1 μM and evaluated over a range of temperatures. **A**) crySI. **B**) crys96. **C**) The cry peptide alone. **D**) CrySI displayed a temperature dependent intensity change, which was significantly greater than for crys96 or cry alone (****p<0.0001). Values indicate the mean ± SD. (n=3, ANOVA, followed by the Bonferroni post-hoc test). **E-F**) When peptides were incubated at 5 μM at a fixed temperature (25 °C), both crySI (~10 fold) and crys96 (~1.7 fold) increased the fluorescence intensity compared to the cry peptide alone (***p<0.001), which suggested enhanced hydrophobicity. Data were presented as mean±S.D. (n=3, ANOVA, followed by the Tukey's post-hoc test).



Supporting Figure S5. Proposed intracellular trafficking and protective mechanism of cryS96 under oxidative stress. When human RPE cells are incubated with cryS96 in the absence of oxidative stress, the peptide partially traffics to lysosomes, as indicated by colocalization with LAMP1 (Fig. 8). In contrast, under H_2O_2 stress the cryS96 translocates to the nucleus (Fig. 6 and Fig. 8) as well as gains access to the cytosol. Having access to the cytosol, the cryS96 peptide halts apoptosis, by blocking caspase 3 activation and inhibiting apoptosis (Fig. 5). Under oxidative stress, both nuclear translocation of cryS96 and anti-apoptosis activity were blocked by a dynamin-dependent mechanism (Supplementary Fig. S3) or inhibited by monodansylcadaverine (Fig. 7). The mechanism by which cryS96 binds to the cell surface, escapes from endosomes under oxidative stress, or blocks the activation of caspase 3 remains unknown.