## Legend Supplementary Fig. S1.

Chromatographic traces of three tryptic peptides from each of $\alpha \mathrm{A}, \alpha \mathrm{Ba}$, and $\alpha \mathrm{Bb}-c r y s t a l l i n s$ from wildtype and cryaa, cryaba, and cryabb mutant lenses from adults. Wild-type and cryaba mutant lenses were 12 months old and cryaa and cryabb mutant lenses were 6 months old. A-C: aA 53-66, 90-100, and 121-146, respectively. D-F: $\alpha$ Ba 80-80, $90-100$, and 155-168, respectively. G-I: $\alpha B b: 12-20,46-58$, and $82-$ 92 , respectively. In each panel A-I: 1: wild-type lens 1, 2: wild-type lens 2,3 : cryaa mutant lens (first generated mutant), 4: cryaa null lens (second generated mutant), 5: cryaba mutant lens 1, 6: cryaba mutnat lens 2,7 : cryabb mutant lens 1,8 : cryabb mutant lens 2 . In each pair of chromatograms, the upper trace shows the peak for the designated peptide precursor ion of the designated charge state, and the lower trace shows three corresponding coeluting peptide $y$ - and b-fragment ions. The intensity scales for each grid of chromatographic peaks were not set at the same scale so that lower intensity peaks could be observed. When peaks were not observable in digests of null lenses, the intensity scale was set at the same value as the lowest intensity scale on chromatograms from lenses where the respective $\alpha$-crystallin was not knocked out. Underlined cysteines denote alkylation by iodoacetamide. Chromatograms were generated using Skyline software and can be accessed on PanoramaWeb (accession number pending).

## A) $\alpha A:$ R.NILDSSNSGVSEVR.S $[53,66]$

1. Wild-type lens 1

2. Wild-type lens 2


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4. cryaa mutant (2nd generated) 6. cryaba mutant lens 2


7: cryabb mutant lens 1



8: cryabb mutant lens 2


## B) $\alpha A: K . V T D D Y V E I Q G K . H[90,100]$



## C) $\alpha$ A: R.LPSNVDQSAITCTLSADGLLTLCGPK.T [121, 146]

## 1. Wild-type lens 1


2. Wild-type lens 2


3. cryaa mutant (1st generated)
5. cryaba mutant lens 1


4. cryaa mutant (2nd generated) 6. cryaba mutant lens 2



7: cryabb mutant lens 1


8: cryabb mutant lens 2


D) $\alpha$ Ba: K.HFSPDELTVK.V [80, 89]

E) $\alpha$ Ba: K.VNEDFIEIHGK.H [90, 100]


## F) $\alpha$ Ba: R.SIPIICGEKPPAQK.- [155, 168]


G) $\alpha$ Bb: R.ILFPIFFPR.R [12, 20]


## H) $\alpha$ Bb: R.SPSWMESGVSEVK.M [46, 58]

$\overline{\mathrm{w}}$ 1. Wild-type lens 1

$\overline{\mathrm{w}}$ 2. Wild-type lens 2


5. cryaba mutant lens 1


4. cryaa mutant (2nd generated) 6. cryaba mutant lens 2



7: cryabb mutant lens 1


8: cryabb mutant lens 2

I) $\alpha$ Bb: K.IIGDFIEIHAK.H [82, 92]

## 1. Wild-type lens 1



2. Wild-type lens 2


3. cryaa mutant (1st generated)


4. cryaa mutant (2nd generated) 6. cryaba mutant lens 2


5. cryaba mutant lens 1



7: cryabb mutant lens 1



8: cryabb mutant lens 2



