Captions for Supplemental Figures and Tables

Figure S1. A 2D-DIGE analysis of proteomic changes in whole lenses of 2-day-old DKO mice. (A) A 2D gel of WT1 lens proteins labeled with Cy3. (B) A 2D gel of DKO1 proteins labeled with Cy2. Arrowheads indicate the position of spots where different forms of αA-insert and αB-crystallin are expected to migrate. Arrows indicate the position where different forms of αA-crystallin are expected to migrate. Note that the αA-crystallin and αB-crystallin bands are absent in the DKO1 gel.

Figure S2. Sequence coverage of protein spots identified as (A) β1-catenin and (B) βB1-crystallin in 2-day-old mouse lenses.

Figure S3. Sequence coverage for proteins identified as (A) vimentin and (B) βB2-crystallin in 1- to 2-month-old mouse lens epithelial fractions.

Figure S4. Ingenuity networks created for proteins whose abundance was changed in DKO mouse lenses as compared with WT. Biological networks and pathways were generated from input data in Tables 1-3. These three networks represent the position of proteins in different cellular networks. See Figure 5 for an additional four networks generated by the data in Tables 1-3.

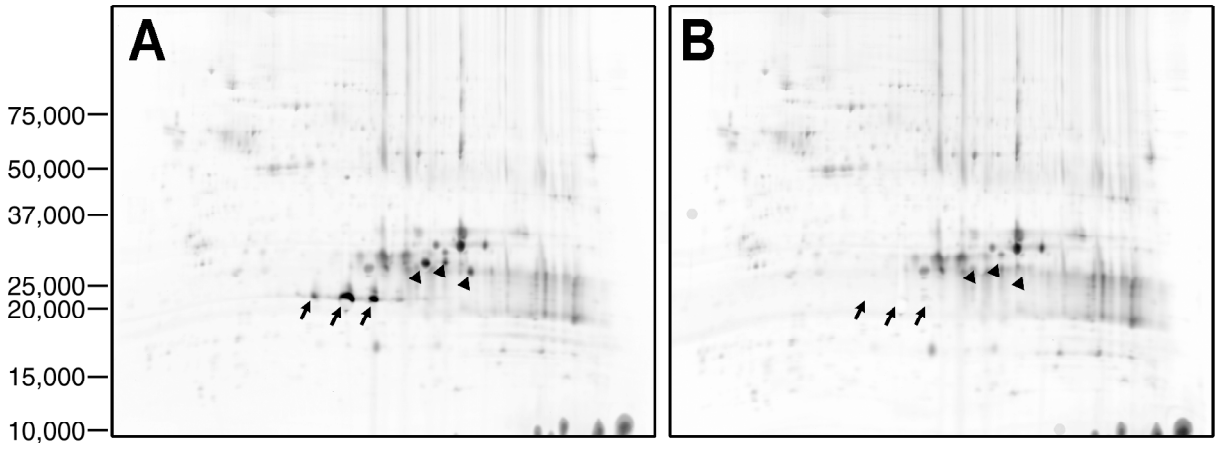
Figure S5. Immunoblot analysis of changes in amounts of βB2-crystallin and vimentin in WT vs. DKO lenses. Lens proteins were resolved by SDS-PAGE and analyzed by immunoblotting with antibodies specific for βB2-crystallin (top) and vimentin (bottom). Molecular weight markers (Markers) are shown on the left. Lane 1, WT water-soluble proteins; lane 2, WT water-insoluble proteins; lane 3, DKO water-soluble proteins; lane 4, DKO water-insoluble proteins.

Figure S6. GPC analysis of equal protein concentrations (200 μg) of water-soluble proteins isolated from WT and DKO lenses. (A) RI profiles for 7-day-old WT and 9-day-old DKO lens proteins. (B) Light scattering profiles for 7-day-old WT and 9-day-old DKO lens proteins. Arrows in A and B indicate new bands observed at 328 kDa in DKO lens proteins. (C) RI profiles for adult WT and adult DKO lens proteins. The arrow indicates a new shoulder observed in the RI profile of adult DKO lens proteins. (D) Light scattering profiles for adult WT and adult DKO lens proteins. The arrow indicates a new light scattering species eluting near the void volume of the DKO lens proteins.

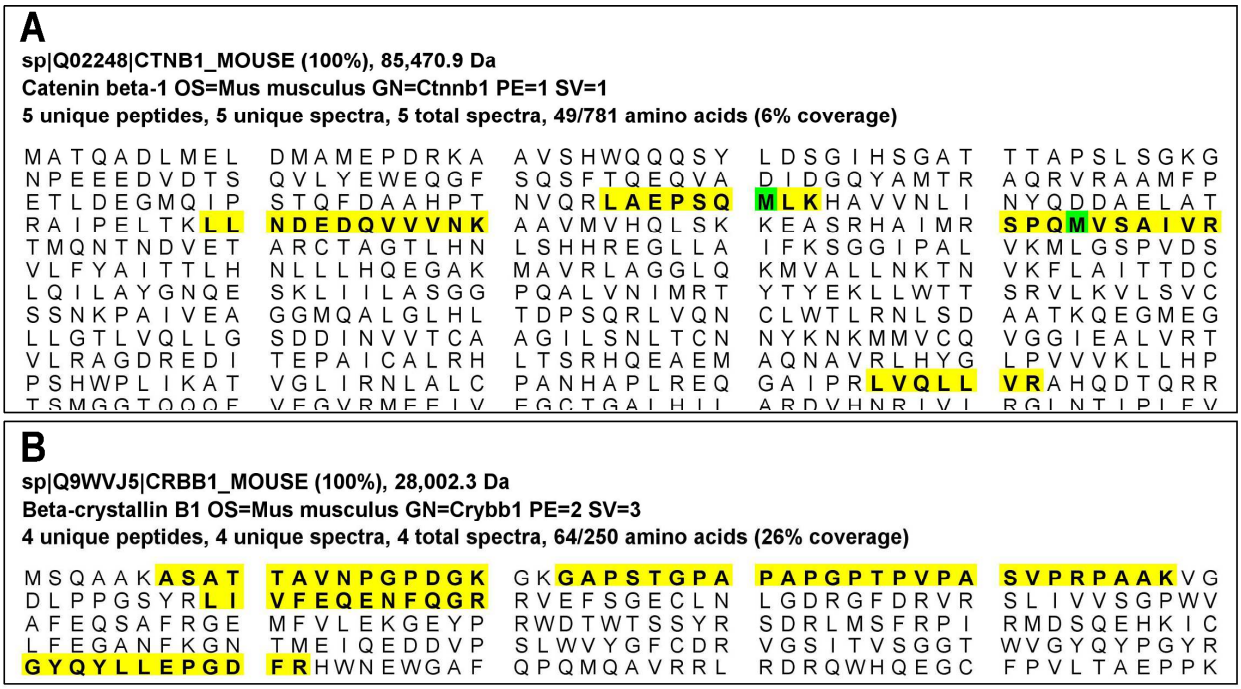
Figure S7. Immunoblot analysis of WT and DKO lens protein fractions. Column fractions were collected from gel permeation chromatography separation of water-soluble lens proteins. Lanes 1-5 indicate fractions eluting at retention volumes of 12, 13, 15, 16, and 18 ml. Note that in 9-day-old DKO lens proteins, a band at 328 kDa was detected with a β-crystallin antibody (lane 2). These fractions did not show reactivity with a γ-crystallin antibody (not shown).

Figure S8. (A) Primers used to assess vimentin and βB2-crystallin transcript levels in the lens. (B) PCR conditions for vimentin and βB2-crystallin real-time PCR. (C) Amounts of βB2-crystallin and vimentin gene transcripts in WT and DKO lenses were measured by quantitative PCR analysis then normalized to that of the GAPDH transcripts. Ten lenses were pooled for each analysis.

Supplemental Figure S1



Supplemental Figure S2



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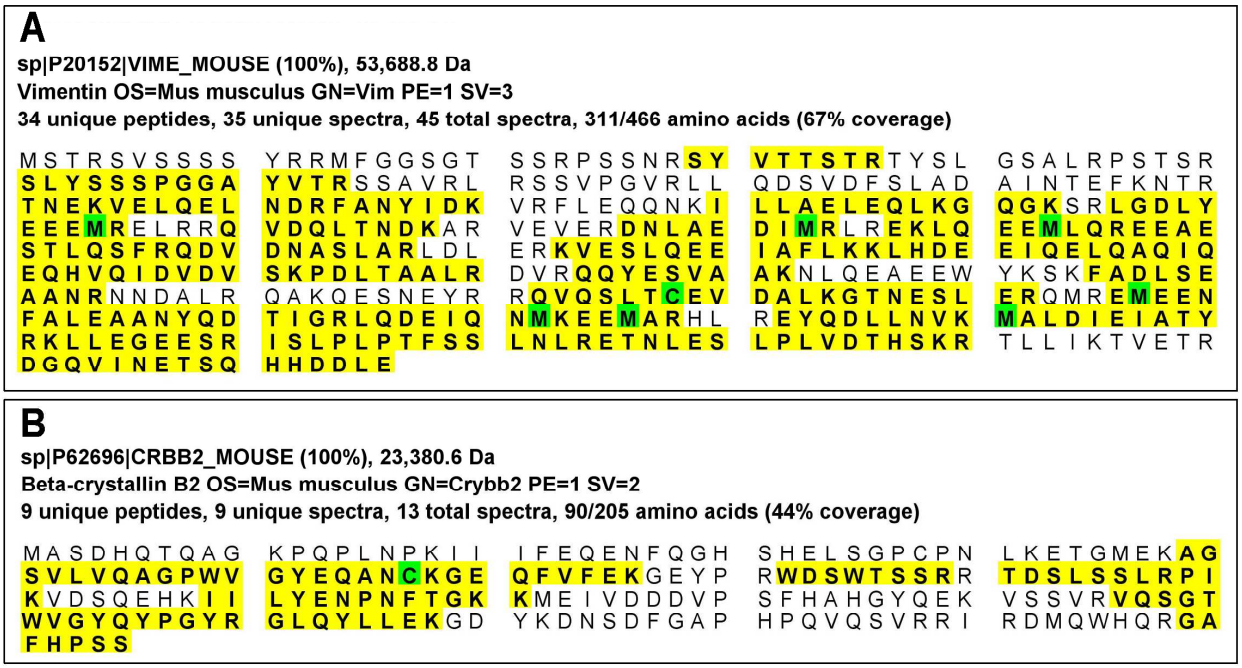

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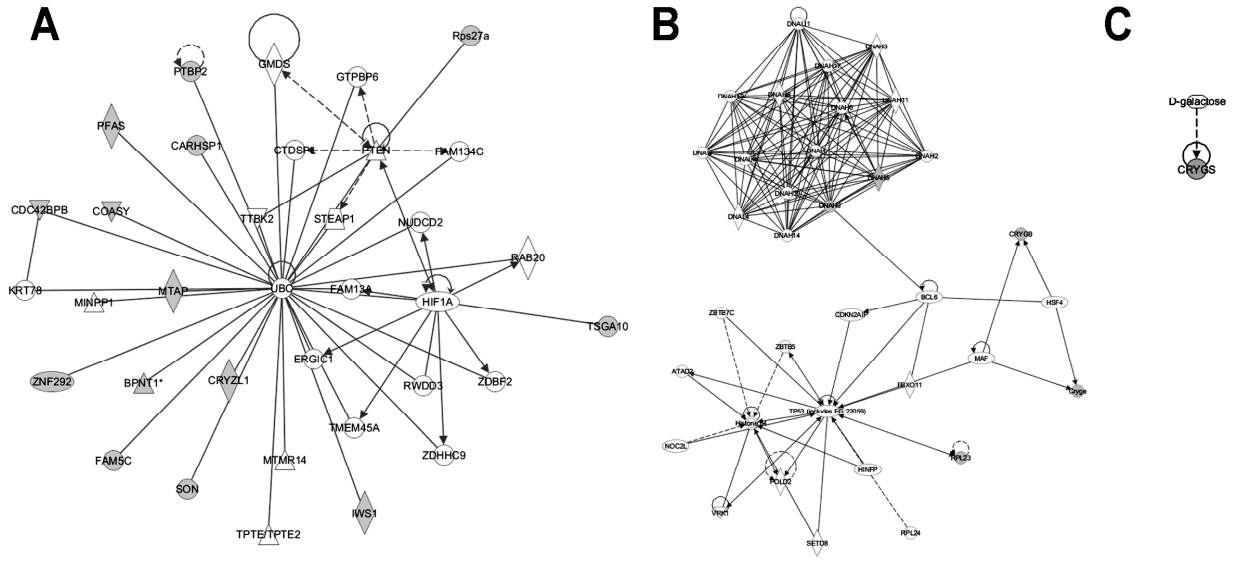
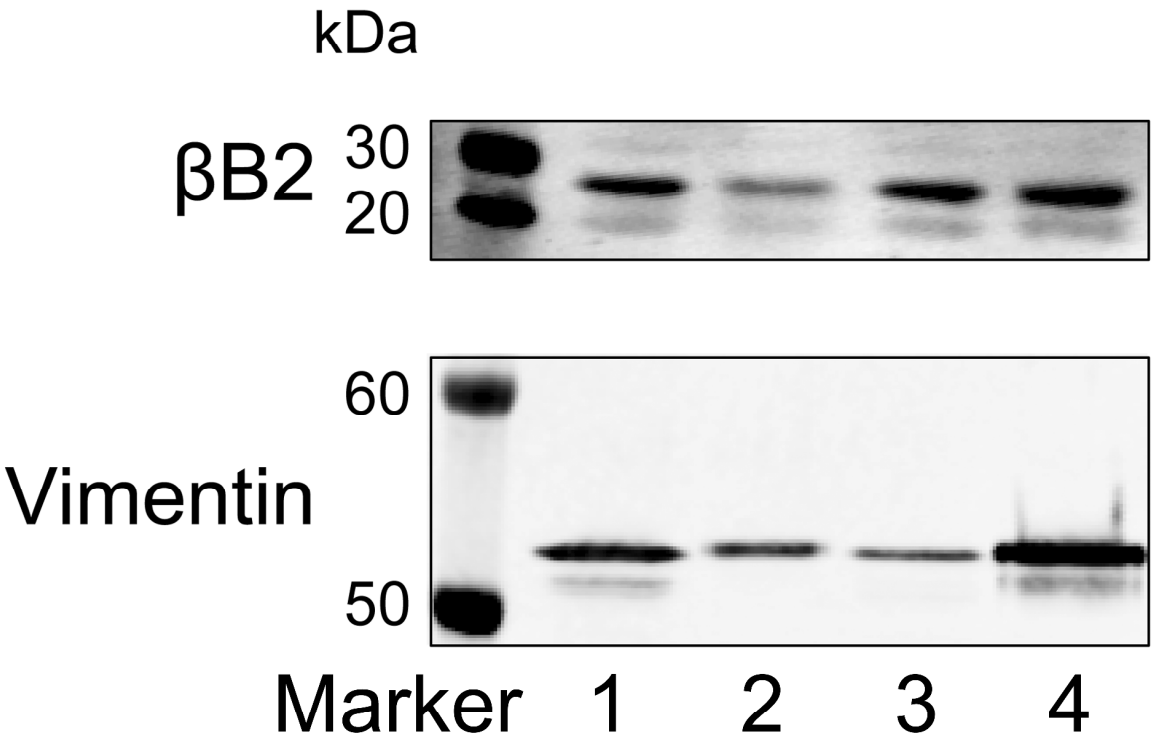
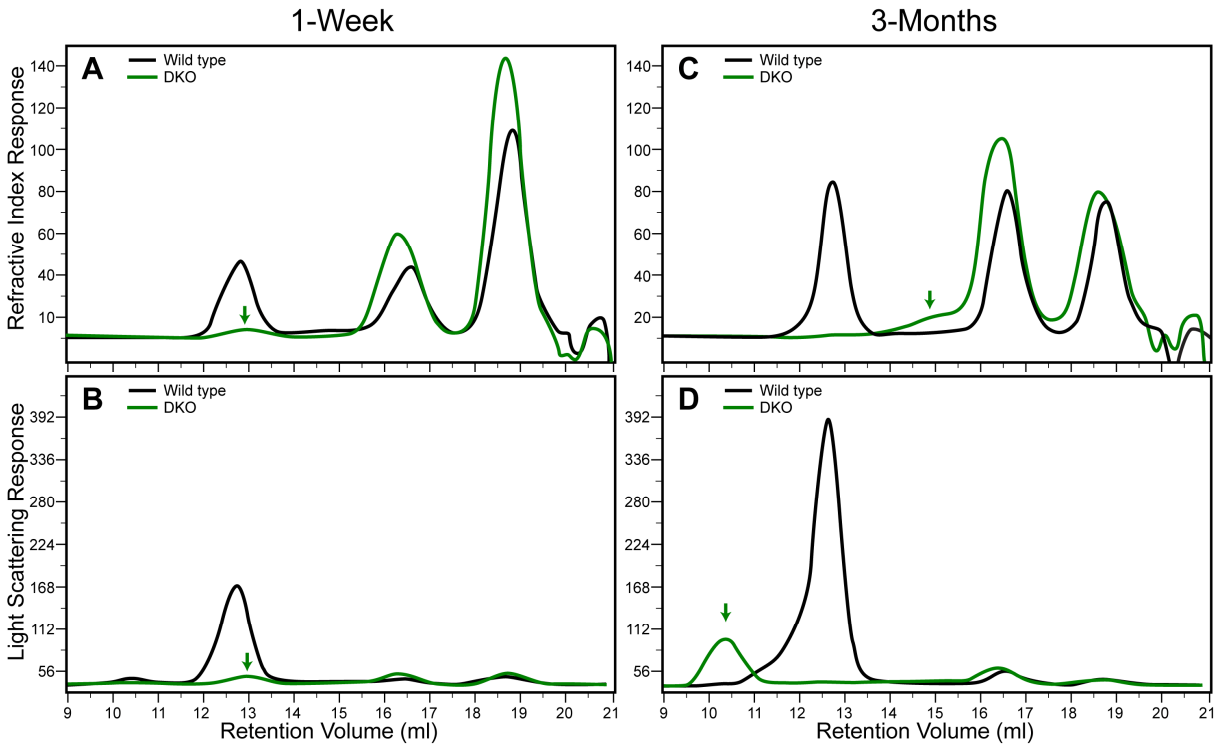


Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7

Supplemental Figure S8

(A) Primers and experimental conditions used in PCR analysis of vimentin and βB2-crystallin in mouse lenses.

Vimentin:
Forward primer 5' -ATC GAT GTG GAC GTT TCC AAG CCT -3'
Reverse primer 5' -ATC CAC TTC ACA GGT GAG TGA CTG -3'
These primers generated a 228 bp PCR product

βB2-crystallin:
Forward primer 5' -AAC AGG AGA ACT TCC AGG GCC ATT -3'
Reverse primer 5' -ACT TTG ATG GGC CTC AGA GAG CTG -3'
These primers generated a 238 bp PCR product

(B) PCR Conditions:

Step 1, 94°C, 2 minutes
Step 2, 94°C, 30 seconds
Step 3, 62°C, 30 seconds
Step 4, 68°C, 1 minute
Step 5, go to step 2, 34 times
Step 6, 68°C, 7 minutes
Step 7, 4°C, ∞

(C)