Supplementary Information

Curve fitting

Methods

The log of the absolute amount of phosphorylation determined for each sample was fit to the following model:

$$\text{Log (Abs. Amt.)} = (A+B-C)^{(-k1(1-r))}-A^{(-k2(1-r))}$$

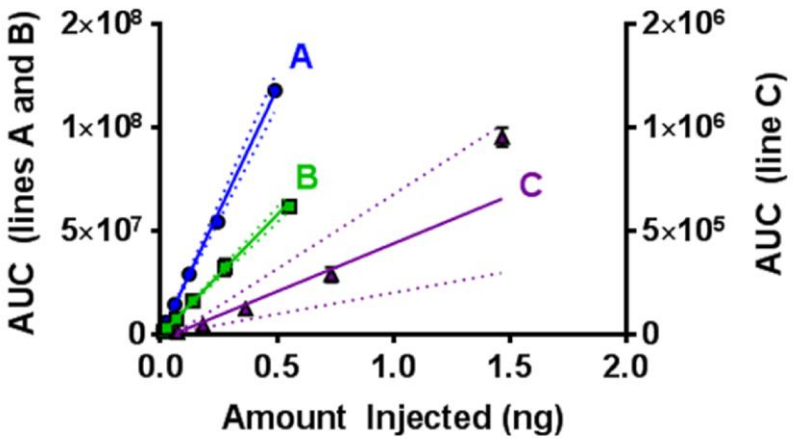
where Abs. Amt. is the mean absolute amount of phosphorylation per sample determined from three replicates, r is the normalized radial distance, k_1 and k_2 are rate constants of growth and decay respectively, and A, B, C are constants that describe, respectively, the peak of the curve, the minimum value at x=1, and the plateau of the curve as x approaches 0. The model expresses the increase in the amount of a product (in this case phosphorylated peptide) from the initial substance (in this case unphosphorylated peptide) and the subsequent decrease in this product either to the initial substance or a degradation product; thus, it represents that which is occurring biologically. Nonlinear regression was used to fit the data for each lens to the above model and determine best fit values for the rate constants, A, B, and C. The data passed the D'Agnostino-Pearson omnibus K2 normality test. Statistical analyses were performed using GraphPad Prism 5 Demo. The sum of squares was minimized via the Marquardt method with the maximum number of iterations set at 10,000, and best fit values for all parameters were constrained to those greater than zero. To ensure that the initial values provided during curve fitting did not excessively influence the best fit values, 10 different sets of best fit values (per lens) were tested for curve fitting. Bounds were placed on the initial values as follows, $k_1 \leq k_2$, $B \leq A$, and $1 \leq k_1, k_2, A, B, C \leq 30$. Additionally, initial values that resulted in curves that greatly deviated from the data points were not used. For datasets that were sensitive to initial values, and thus had several potential sets of best fit values, a single set of best fit values was selected after taking into account the absolute sum of squares, the confidence intervals, and the R^2 value.

Results

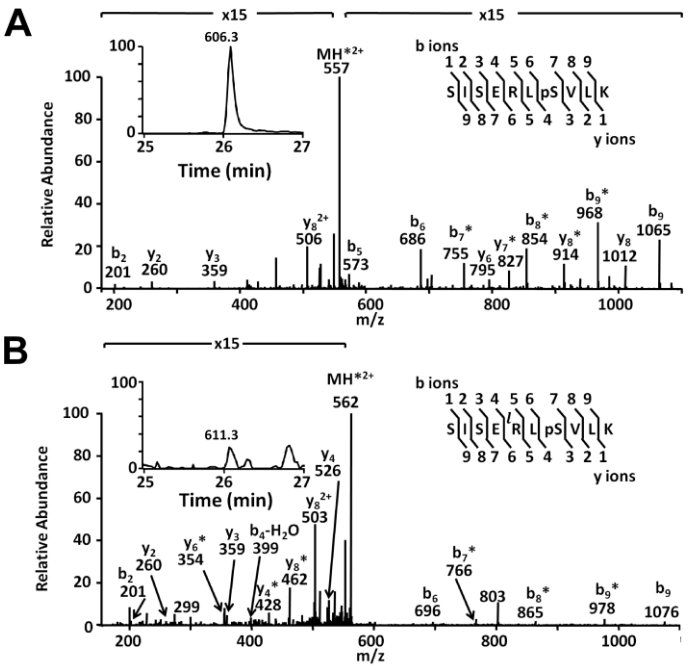
Best fit values determined for MP20 pS170 from the 18 year lens and AQP0 pS235 data from the 18 and 23 year lenses were not sensitive to the initial values

provided during curve fitting. The other lenses were sensitive to the initial values provided, but only in a minor way, such that the combined parameters for one fit did not alter the shape of the curve obtained using parameters from a different fit. This was confirmed quantitatively by obtaining values of Log(Abs. Amt) for a range of x values (.95-0.7, in increments of 0.01 and 0.65-0 in increments of 0.05) for each set of best fit values determined by the corresponding set of initial values. The maximum difference in Log(Abs. Amt) across the 10 sets of values was as follows, 0.06 (19 yr MP20), 0.05 (23 yr MP20), 0.00 (51 yr MP20), 0.01 (54 yr MP20), 0.03 (60 yr MP20), 0.01 (19, 51 yr AQP0), 0.00 (54, 60 yr AQP0). These differences are small and show that despite the potential for different best fit values, the shape of the curve, and therefore the resulting value of interest, Log(Abs. Amt), does not change greatly. The largest change was for the 19 yr MP20 data, which was 0.06 at r = 0-0.05. This corresponds to an absolute difference of 13 fmol (max = 1.78, min = 1.51), which represents 16% of the total amount (80 fmol). Importantly, the amounts of phosphorylation in the older and younger lenses differ significantly in this region. Therefore, it can be concluded that the dependence on initial starting value is not sufficient to cause great differences in the resulting amounts of phosphorylation.

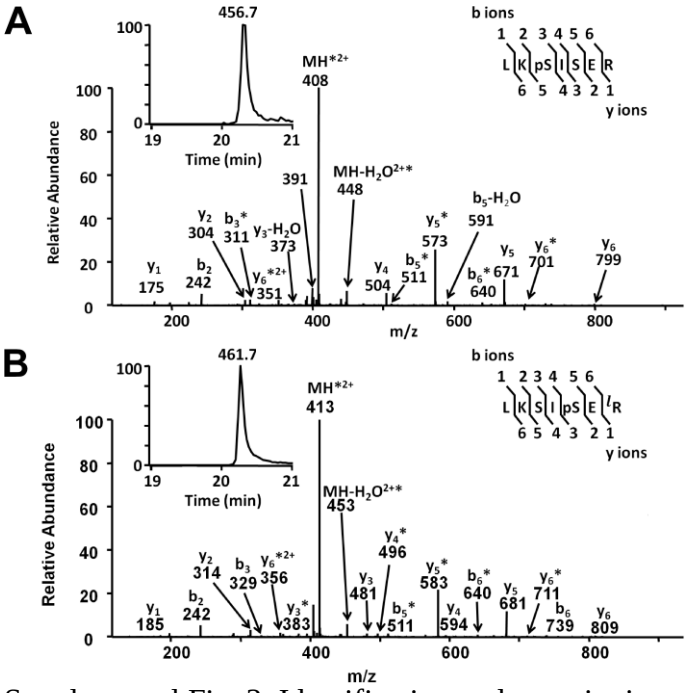
Supplemental Figures



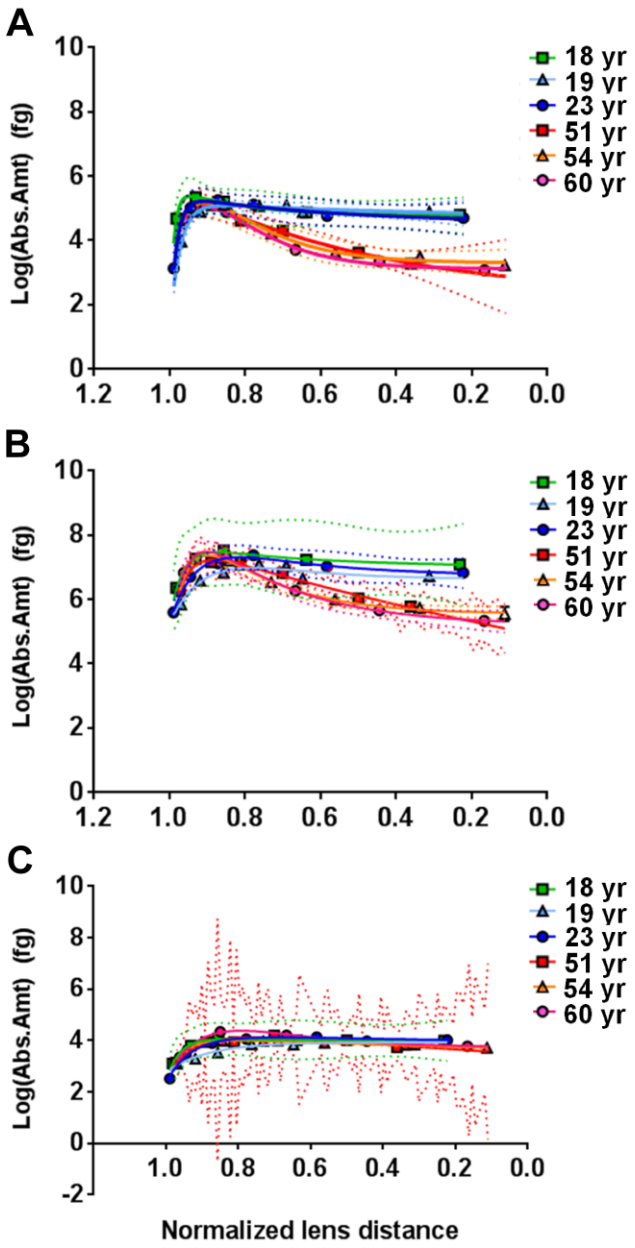
Supplemental Fig. 1. AQUA peptide standard curves. Standard curves were generated with $1/y^2$ weighting for AQUA peptide internal standards, A) MP20 168–173 pS170 ($r^2 = 1.0$), B) AQP0 227–233 pS231 ($r^2 = 1.0$), and C) AQP0 229–238 pS235 ($R^2 = 0.92$). Dashed lines correspond to the 95% confidence bands for each line.



Supplemental Fig. 2. Identification and quantitation of AQP0 229–238 pS235. MS/MS spectra from a 23 year lens confirmed the identity of A) endogenous AQP0 229–238 pS235 and B) its isotopically labeled AQUA peptide internal standard. Within the MS/MS spectra, the asterisks represent loss of phosphoric acid. The peptide sequence and fragmentation are shown in the top right corner of each panel, where phosphorylation is indicated by "p" and the isotopically labeled amino acid is indicated by "***I***" (bottom panel only). The XICs of AQP0 229–238 pS235 (panel A, insert) and its AQUA internal standard (panel B, insert) show the difference in mass between the $(M+2H)^{2+}$ ions for the endogenous and labeled peptides and the identical elution times of these peptides.



Supplemental Fig. 3. Identification and quantitation of AQP0 227–233 pS229. MS/MS spectra from a 60 year lens confirmed the identity of A) endogenous AQP0 227–233 pS229 and B) the isotopically labeled AQUA peptide internal standard, AQP0 227–233 pS231. Within the MS/MS spectra, the asterisks represent loss of phosphoric acid. The peptide sequence and fragmentation are shown in the top right corner of each panel, where phosphorylation is indicated by "p" and the isotopically labeled amino acid is indicated by "***I***" (bottom panel only). The XICs of AQP0 227–233 pS229 (panel A, insert) and its AQUA internal standard AQP0 227–233 pS231 (panel B, insert) show the difference in mass between the $(M+2H)^{2+}$ ions for the endogenous and labeled peptides and the identical elution times of these peptides. Note, the majority of the endogenous phosphorylation was determined to reside at Ser229; however, low intensity signals were seen that could indicate a small portion of this peptide was phosphorylated at Ser231.



Supplemental Fig. 4. Distribution of lens membrane phosphopeptides. Curves were fit to the model Log(Abs. Amt.) = (A + B−C)*$e^{(-k_1(1-r))}$−A*$e^{(-k_2(1-r))}$+C using nonlinear regression for A) MP20 168–173 pSer170, B) AQP0 229–238 pSer235, and C) AQP0 227-223 pS229. The values graphed here represent the amount of phosphorylated peptide measured per sample. The amount of phosphorylated peptide per picogram of lens membrane protein (as stated in the text) can be determined by dividing these values by 42,183 pg. Color coded dashed/dotted lines represent the 95% confidence bands for each curve. For additional details see Figs. 2–4, which correspond to A–C respectively.