

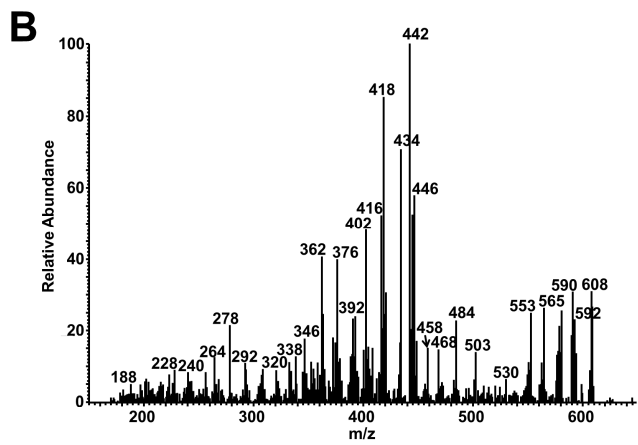
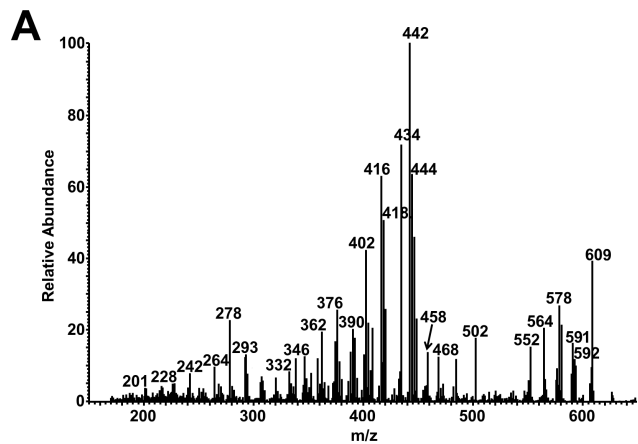
picomoles per eyecup determined for each sample. **C**) Representative peaks from which the AUC (shaded regions) was determined for quantitation of A2E from *ABCR*^{-/-} samples via HPLC (left) or mass spectrometry (right). Samples were analyzed in triplicate. Error was calculated as standard deviation.

Figure 5. Relative quantitation of oxidized A2E. Extracted ion chromatograms from LC-MS/MS analysis of **A**) 100 fmol of synthetic A2E and **B**) approximately 1000 fmol of A2E from a diluted A2E HPLC fraction from an *ABCR*^{-/-} mouse (12 months of age) eyecup preparation were used to determine the area under the curve (AUC) for unoxidized A2E ($m/z = 592$, top panel of A and B) and singly-oxidized A2E ($m/z = 608$, bottom panel of A and B). **C**) Relative quantitation of oxidized A2E was determined by comparing the AUC for oxidized A2E to the combined AUC for oxidized and unoxidized A2E. Numbers on top of the bars represent the percent of singly-oxidized A2E from samples containing synthesized A2E or A2E obtained from the *ABCR*^{-/-} samples. Error was calculated as standard deviation.

Figure 6. Oxidation of A2E occurs during sample handling. **A**) MS/MS spectrum of singly-oxidized A2E ($m/z = 608$) from 100 fmol of synthetic A2E standard. Asterisks indicate fragment ions for which potential structures are shown in panels B-D. As the ions present in the tandem mass spectrum can be produced when the oxygen is in three different locations, the fragmentation pattern of singly-oxidized A2E supports three sites of oxidation, **B**) a 7, 8 epoxide, **C**) a 5, 8 monofuranoid, and **D**) a 9, 10 epoxide. In these structures, placement of the oxygen on the short arm of A2E was done for simplicity; it is likely that the oxygen also occurs on the long arm.

Supplementary Figure 1. Oxidation of A2E occurs during sample handling. **A**) A tandem mass spectrum of oxidized A2E ($m/z = 608$) from a diluted (50 fmol/ μ L) A2E HPLC fraction from an *ABCR*^{-/-} mouse (12 months

of age) eyecup preparation. **B)** A tandem mass spectrum of oxidized A2E ($m/z = 608$) from a diluted (50 fmol/ μL) A2E HPLC fraction from a 94-yr-old human eyecup sample.



Supplementary Figure 1.