

Supplementary Methods and Figure Legends

Methods

A 19 year old and a 22 year old lens were used to further confirm the truncation sites of connexins. The lenses were attached to a chuck with optimal cutting temperature (OCT) medium (Tissue-Tek®, Sakura Finetek, Torrance, CA). Lenses were sectioned equatorially in a cryostat (LEICA CM 3050S) at -21°C . 30 μm sections were cut through the whole lens. Only sections from the equatorial region were collected. Sections were placed on a piece of parafilm and saved on dry ice. Each section, on parafilm, was dried at room temperature and different regions of the lens were isolated by punching through the parafilm using AcuPunch (Acuderm Inc, Ft. Lauderdale, FL) tissue punches. The inner nucleus region was obtained by punching the middle of the section using a 4.5 mm diameter AcuPunch. Further punching the remaining section with a 6 mm diameter punch gave the outer nucleus region. The remaining material was collected as cortex. Sample preparation for LC-MS/MS analysis was processed identically as described in the methods section of the manuscript.

Figure Legends

Figure S1: Truncation sites identified in C-terminus of Cx46 and Cx50 in 19 and 22 year old lenses. Cx46 and Cx50 truncation sites in the different regions of the lenses are identical to those found in older 55 year old lenses shown in Figure 2 of the manuscript. The only truncation site that was not detected in these younger lenses was at A250 in Cx46. In Cx50, the level of truncation at cytoplasmic tail 274-289 region does not increase from cortex to nucleus region. Further truncation of the protein in this region may result in the decreased level of truncated peptides identified in the inner nucleus. A continuous increase of truncation in the cytoplasmic tail 238-253 region can be detected in the younger lenses.

Figure S2: Truncation sites identified in Cx46 and Cx50 cytoplasmic loops in 19 and 22 year old lenses. Cx46 and Cx50 truncation sites in the different regions of the lenses are identical to those found in older 55 year old lenses shown in Figure 3 of the manuscript. Truncation in the cytoplasmic loop of Cx46 and Cx50 increases from cortex to the nucleus, similar to older lenses. However, the levels of the truncations are substantially lower than in the older lenses. In this figure, the level of truncation in the cytoplasmic tail region was normalized to peptide 118-123 because the missed cleavage peptide 118-133 was not detected in these samples.

Figure S3-S8: Tandem mass spectra of the abundant truncated Cx46 and Cx50 peptides