# The supplementary information included the following figures and tables

**Table S1:** The comparison of phosphorylated peptides detection before and after CIP treatment.

**Figure S1:** MALDI MSI of phosphorylated peptide from α-casein (VPQLEIVPNSPAEER) without normalization (a, b) and with normalization by matrix ion (c, d), standard peptide (e, f) and standard phosphophopeptides (g, h) on-target (upper panel) and on-tissue (lower panel).

**Table S2.** Peptides information and MSI before and after the normalization by DHB.

**Figure S2:** Mass spectrum of lens tissue slice obtained by on-plate digestion with GO-IMER and dephosphorylation with CIP. The matrix DHB peak was labelled with pentagram.

**Figure S3.** Mass spectra of lens tissue slice obtained by on-plate digestion with GO-IMER (a) before dephosphorylation with CIP and (b) after dephosphorylation with CIP.

**Figure S4.** MS/MS spectra of (a) AP<u>S</u>WFDTGLSEMR (the underline indicates the phosphorylated site) from alpha-crystallin B and <u>T</u>LGPFYPSR from alpha-crystallin A.

**Figure S5**. The comparison of phosphorylated peptides distribution between normal and cataractous lenses before and after CIP treatment.

**Figure S6.** The comparison of non-phosphorylated peptides ( from beta-crystallin B3) distribution between normal and cataractous lenses before and after CIP treatment.

**Table S3.**: Summary of phosphorylated proteins and peptides of human lenses.

**Table S1.** The comparison of phosphorylated peptides detection before and after CIP treatment.

| Protein | β-casein | ovalbumin | α-casein | |
|---|---|---|---|---|
| Phosphorylation Site | RELEELNVPGEIVESLSSSEESITR | EVVGSAEAGVDAASVSEEFR | VPQLEIVPNSAEER | YKVPQLEIVPNSAEER |
| | PHOS: 30, 32,33,34 | PHOS: 345 | PHOS: 130 | PHOS: 130 |
| S/N before CIP treatment | 64 | - | 48 | - |
| S/N after CIP treatment | 640 | 55 | 256 | 436 |

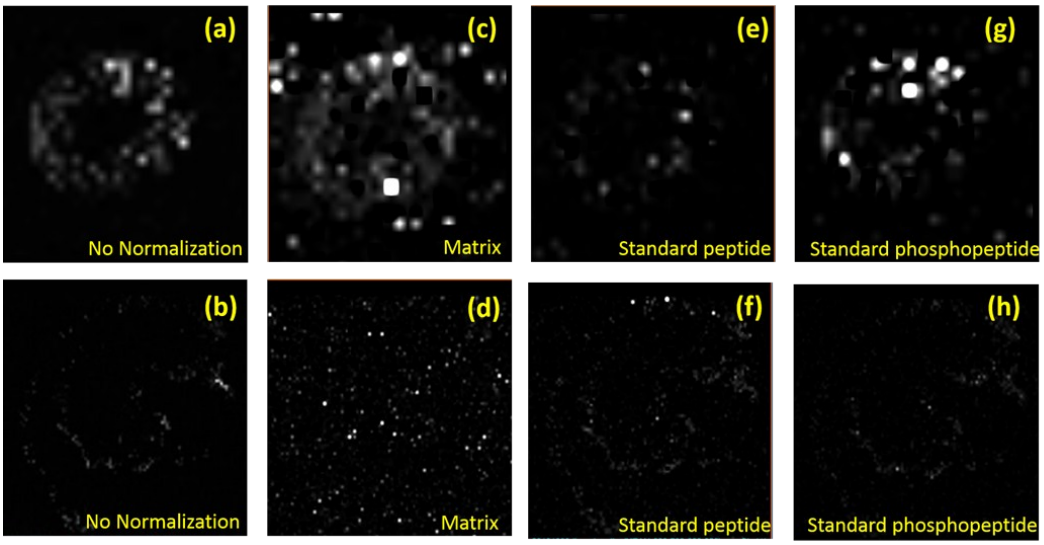
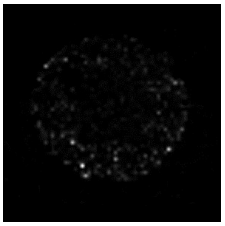
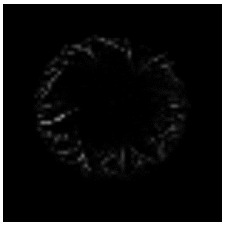
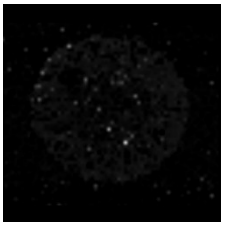
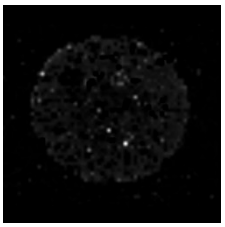



**Figure S1.** MALDI MSI of phosphorylated peptide from α-casein (VPQLEIVPNSPAEER) without normalization (a, b) and with normalization by matrix ion (c, d), standard peptide (e, f) and standard phosphophopeptides (g, h) on-target (upper panel) and on-tissue (lower panel).

**Table S2**. Peptides information and MSI before and after the normalization by DHB.

| Amino acid sequence | m/z | Gravy | Before normalization | After normalization by DHB peak |
|---|---|---|---|---|
| EDVPSER | 831.38 | -1.886 |  |  |
| YLGYLEQLLR | 1267.70 | 0.070 |  |  |
| HQGLPQEVLNENLLR | 1759.94 | -0.753 |  |  |

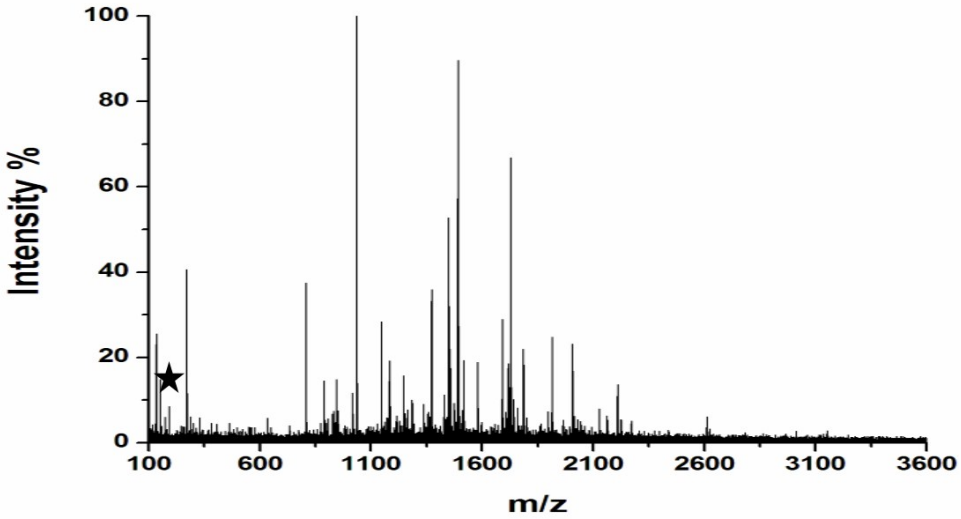



**Figure S2**. Mass spectrum of lens tissue slice obtained by on-plate digestion with GO-IMER and dephosphorylation with CIP. The matrix DHB peak was labelled with pentagram.

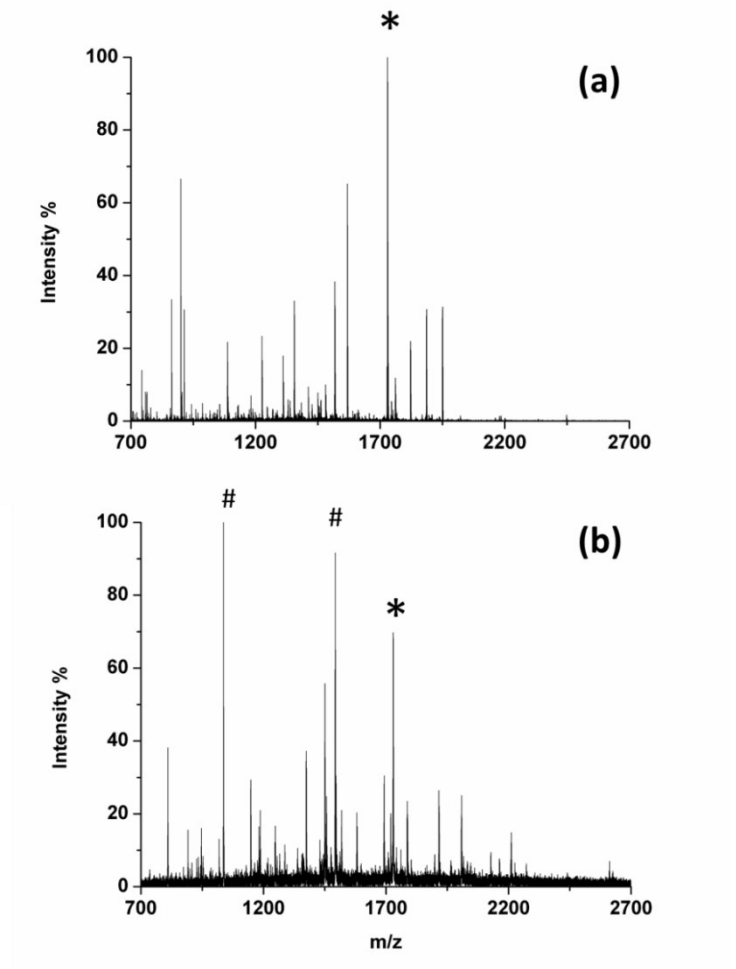



**Figure S3.** Mass spectra of lens tissue slice obtained by on-plate digestion with GO-IMER (a) before dephosphorylation with CIP and (b) after dephosphorylation with CIP. * indicates the non-phosphorylated peptide (AINGTWVGYEFPGYR, m/z 1729.28). # indicates dephosphorylated peptides (TLGPFYPSR, m/z 1037.13 and APSWFDTGLSEMR, m/z 1496.68).

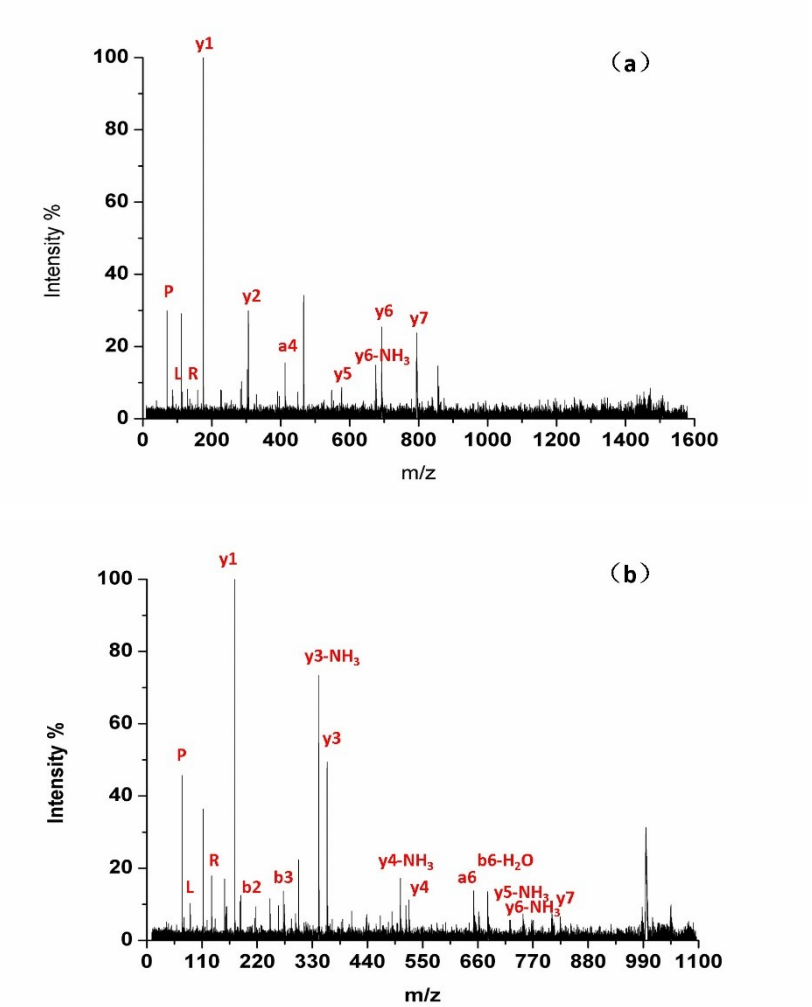



**Figure S4.** MS/MS spectra of (a) APSWFDTGLSEMR (the underline indicates the phosphorylated site) from alpha-crystallin B and TLGPFYPSR from alpha-crystallin A.

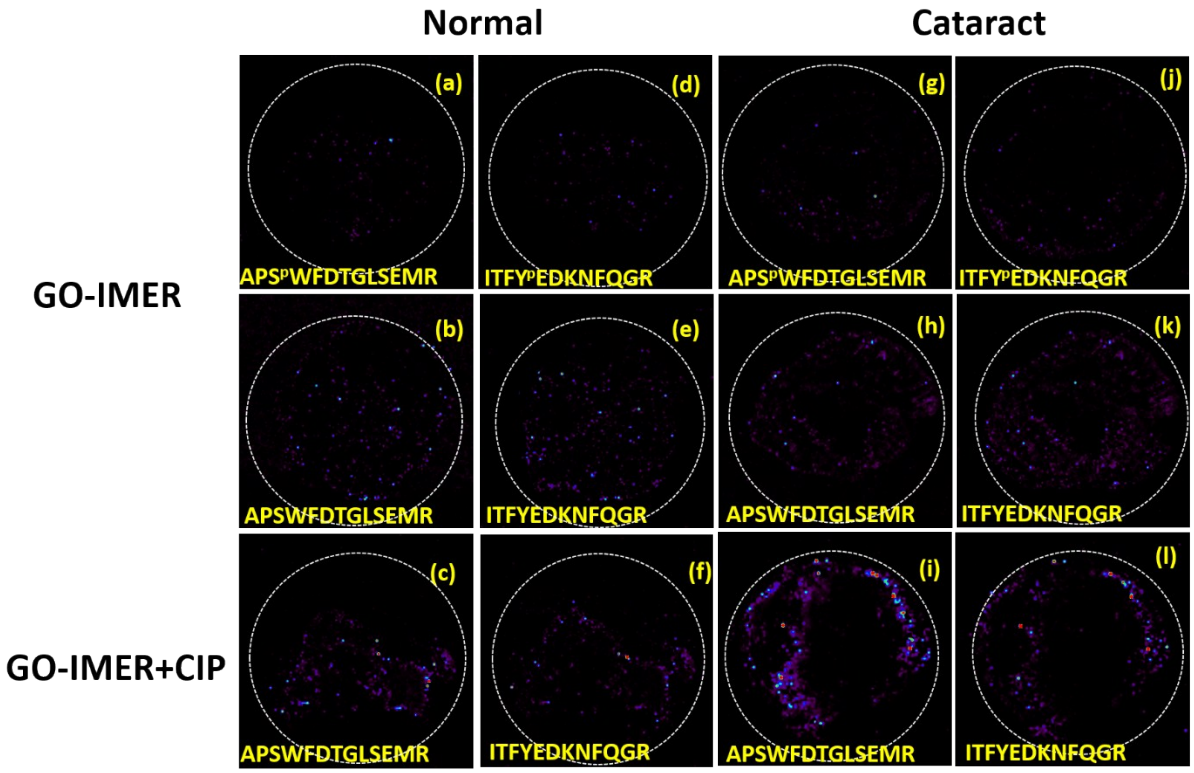



**Figure S5**. The comparison of phosphorylated peptides distribution between normal and cataractous lenses before and after CIP treatment. (a)(b)(c)(g)(h)(i) display the map of a phosphorylated peptide from α-B (APSWFDTGLSEMR, the phosphorylation site was underlined, the same below); (d)(e)(f)(j)(k)(l) display the map of a phosphorylated peptide from β-S (ITFYEDKNFQGR). The left two columns exhibit the peptides distribution of normal human lenses. The right two columns exhibit the peptides distribution of cataractous human lenses. The first line shows the distribution of peptides with phosphate group modification; the second line shows the distribution of peptides without phosphate group before CIP treatment; the third line shows the distribution of peptides without phosphate group after CIP treatment. Peptide amino acid sequence was shown in the bottom left corner.

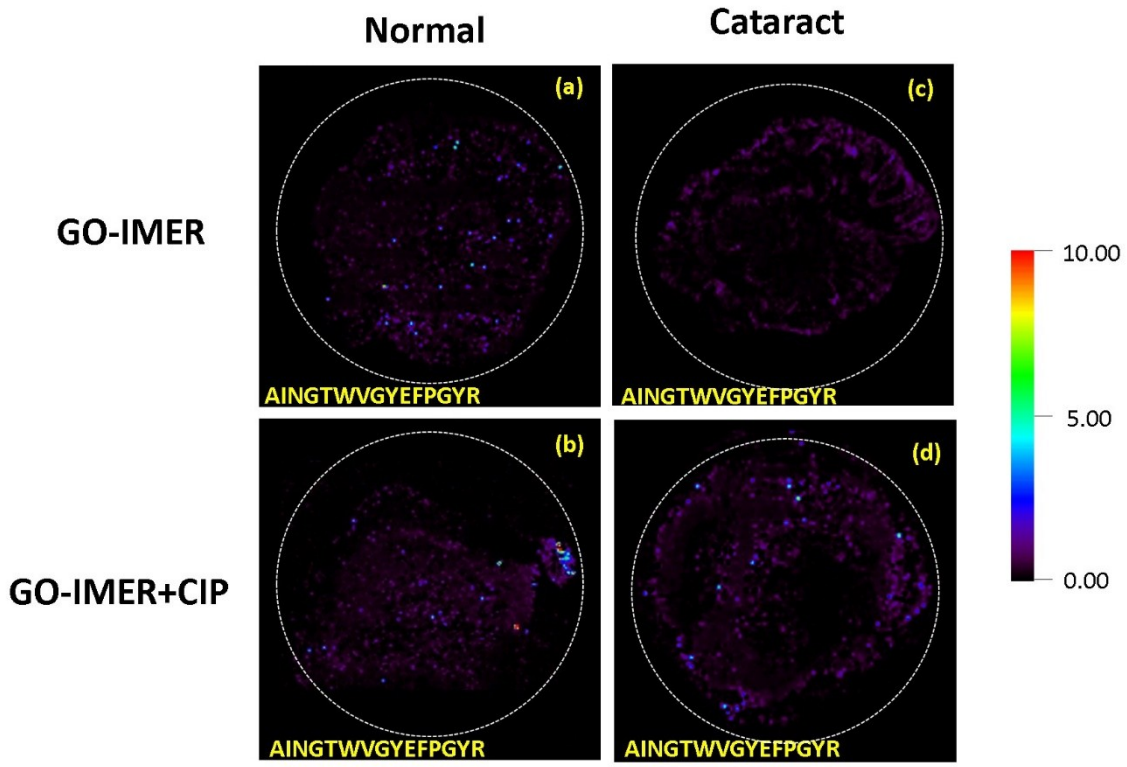



**Figure S6.** The comparison of non-phosphorylated peptides ( from beta-crystallin B3) distribution between normal and cataractous lenses before and after CIP treatment. The left column exhibits the peptides distribution of normal human lenses. The right two column exhibits the peptides distribution of cataractous human lenses. The first line shows the distribution of the peptide before CIP treatment; the second line shows the distribution of the peptide after CIP treatment. Peptide amino acid sequence was shown in the bottom left corner

**Table S3.** Summary of phosphorylated proteins and peptides of human lenses.

| | Protein | Position | Peptide Sequence (the phosphorylation site was underlined) | m/z | Phosphorylated Site |
|---|---|---|---|---|---|
| 1 | Alpha-crystallin A | 13-21 | TLGPFYPSR | 1037.56 | Thr-13 |
| 2 | | 12-21 | RTLGPFYPSR | 1193.68 | Thr-13 |
| 3 | Alpha-crystallin B | 75-82 | FSVNLDVK | 921.54 | Ser-76 |
| 4 | | 73-82 | DRFSVNLDVK | 1191.20 | Ser-76 |
| 5 | | 12-22 | RPFFPFHSPSR | 1374.72 | Ser-19,21 |
| 6 | | 57-69 | APSWFDTGLSEMR | 1496.74 | Ser-59 |
| 7 | | 70-82 | LEKDRFSVNLDVK | 1562.88 | Ser-76 |

| | | | | | |
|---|---|---|---|---|---|
| 8 | | 75-90 | FSVNLDVKHFSPEELK | 1888.99 | Ser-76 |
| 9 | | 57-72 | APSWFDTGLSEMRLEK | 1866.86 | Ser-59 |
| 10# | | 75-92 | FSVNLDVKHFSPEELKVK | 2116.24 | Ser-76 |
| 11 | Beta-crystallin B2 | 109-120 | IILYENPNFTGK | 1408.64 | Thr-118 |
| 12 | | 109-121 | ILYENPNFTGKK | 1423.75 | Thr-118 |
| 13 | | 102-120 | VDSQEHKIILYENPNFTGK | 2232.12 | Thr-118 |
| 14 | Beta-crystallin B1 | 7-22 | ASASATVAVNPGPDTK | 1485.75 | Ser-10 |
| 15 | | 7-24 | ASASATVAVNPGPDTKGK | 1670.87 | Ser-10 |
| 16 | Beta-crystallin A3 | 197-211 | EWGSHAQTSQIQSIR | 1727.84 | Ser-200 |
| 17 | | 197-212 | EWGSHAQTSQIQSIRR | 1883.94 | Ser-200 |
| 18 | Beta-crystallin A4 | 104-118 | DSRLTIFEQENFLGK | 1796.91 | Thr-108 |
| 19 | Beta-crystallin S | 4-14 | TGTKITFYEDK | 1302.65 | Thr-6 |
| 20 | | 8-19 | ITFYEDKNFQGR | 1517.73 | Tyr-11 |
| 21 | | 85-95 | AVHLPSGGQYK | 1156.61 | Ser-90 |
| 22 | Gamma-crystallin D | 154-163 | YQDWGATNAR | 1181.53 | Thr-160 |
| 23 | | 153-163 | RYQDWGATNAR | 1337.63 | Thr-160 |

# denotes detected phosphorylated peptides after CIP treatment.