Deletion of *Alox15* improves kidney dysfunction and inhibits fibrosis by increased PGD$_2$ in the kidney

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Supplementary Methods

Cell culture

Rat kidney epithelial cells (NRK-52E cells) were cultured in Dulbecco's modified eagle medium (4.5 g/L glucose) (Nacalai Tesque) supplemented with 5% fetal bovine serum, whereas human renal proximal tubule cells (HK-2 cells) were cultured in DMEM/F12 (Gibco) supplemented with 10% fetal bovine serum. Both NRK 52E cells and HK-2 cells were purchased from ATCC. We seeded these cells on 6-well culture plates to 80% confluence in a complete medium for 24 h and then transferred them to a serum-free medium. Then, they were preincubated with lipid metabolites for 30 min, followed by the treatment of recombinant human TGF-β1 (Pepro Tech) at 5 ng/mL and the lipid metabolites for different dosage as indicated for 24 h. We cultivated all cells at 37 °C under 5% CO₂ condition in a humidified incubator.

Immunoblotting

We extracted protein samples from the kidneys or cultured cells and performed semiquantitative immunoblotting as described previously [1]. We analyzed and quantified the relative intensities of the immunoblot bands through the ImageJ software (National Institutes of Health). We used primary antibodies such as anti-GAPDH (Santa Cruz Biotechnology, #sc32233, 1:1000), anti-ALOX15 (abcam, #ab244205, 1:1000), anti-NGAL (R&D Systems, #AF1857, 1:1000), anti-fibronectin (abcam, #ab2413, 1:1000) and anti-αSMA (abcam, #ab5694, 1:1000) for the experiment. For secondary antibodies, we used the alkaline phosphatase-conjugated anti-rabbit IgG antibody (Promega, #S3738, 1:1000), anti-goat IgG antibody (Promega, #V115A, 1:1000) and anti-mouse IgG antibody (Promega, #S3721, 1:1000).
Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Total RNA extracted from the mouse kidneys was reverse-transcribed using ReverTra Ace (TOYOBO), and the qRT-PCR analysis was performed in the Thermal Cycler Dice Real Time System (Takara Bio). The primers and templates were mixed with SYBR Premix Ex Taq II (Takara Bio). Thereafter, the mRNA contents were normalized to GAPDH and then calculated using the comparative CT method. Sequence-specific primers for mice, NRK-52E cells and HK-2 cells are listed below.

Sequence-specific primers for mice

|   | Species | Forward 5'→3' | Reverse 5'→3' |
|---|---------|---------------|---------------|
| Gapdh | mouse | CGTGAGTGCTACTGGTGCTTACAC | CGGAGATGATGACCCCTTTTGCC |
| Alox15 | mouse | GAAGACTCTCAAGCCCTGTT | GTCAGAGATACTGGTCGCC |
| Alox5 | mouse | ACTATCTACATCCACCTGCCATT | GTGGACATCGTAGGAGTCAC |
| Ptgs1 | mouse | GGTGCGCCTCACCAGTCATTC | ATTCGCAAGGACGTTCCAAG |
| Ptgs2 | mouse | TGGGGGAGAAATGTGGGCAAC | CAGCATTTCCTTTCTCTCTGT |
| Cyp4a12a | mouse | GGACTTCTATCACCTGGGAAAT | ACTGTTACAGAGGAGGTGAT |
| Cyp2c44 | mouse | CCCGTCTCTGTCTCCATCCTT | GTCTGGATCAAACCTCTCTGG |
| Ptgds | mouse | CGGCTCAATCTCACTCCATCAC | CCTTGTCGCTCTGCTGAAT |
| Hpgds | mouse | TGAAGCTGATGCAGTGATGG | GAAGGCGAGGTGCTGATG |
| Hpjd | mouse | AGCACCGCATCAGCGATT | GTCCCAAAGCCCTGGGCAAC |
| Akr1e18 | mouse | GCCAGGCGATTCTAAAGCAAGA | CCTCACGTGCTTACAGACAC |
| Col1a1 | mouse | TGACTGGAAGAGCGCAAGTGT | GTGCGGGGCTGATGTACCAGT |
| Fn | mouse | AGACTGCACTGACCCCATC | AATGTGCTCCTGAGGAGCAT |
| Acta2 | mouse | GCTGCTCCAGCTATGTGTGA | CATTCCAACCATTACTCCCTGA |

Sequence-specific primers for NRK-52E cells

|   | Species | Forward 5'→3' | Reverse 5'→3' |
|---|---------|---------------|---------------|
| Gapdh | rat | CTGACCAACCAACTGCTTAG | TCAGCTCGGATGACCTTG |
| Col1a1 | rat | TCGAGTATGGAGCGAAGGT | TTGAGGTTGACGCTTGTG |
| Acta2 | rat | ACTGGGACGACATGGAAAAG | GCCACATCAGGGAGAGCATG |
Sequence-specific primers for HK-2 cells

| Species | Forward 5'→3' | Reverse 5'→3' |
|---------|--------------|--------------|
| Gapdh   | human        | ACCAAATCCGTTGACTCCGAC | CTCCTGTTCGACAGTCAGCC |
| Colla1  | human        | GATTCCCTGGACCTAAAGGTGC | AGCCTCTCCATCTTTGCCAGCA |
| Acta2   | human        | ATCAACCAACTGGGACGACAT | GGCAACACGAAGCTCATTG |

**Histological analysis**

We fixed the mouse kidneys in 10% formalin neutral buffer solution (Wako) and histologically analyzed them by using the Masson’s trichrome method as described previously [1].

**In situ hybridization**

The kidneys were fixed by perfusion with periodate lysine (0.2 M) and paraformaldehyde (2%) in phosphate-buffered solution. The fixed samples were embedded in an optimum cutting temperature compound (Tissue Tek) and then cryosectioned (5 µm thickness). RNA in situ hybridization was performed using the RNAscope 2.5 HD Reagent Kit–BROWN (Advanced Cell Diagnostics, #322300) according to the manufacturer’s instructions. We used the Target Probe Mm-Alox15 (Advanced Cell Diagnostics, #539781) for Alox15.

**LC-MS/MS-based mediator lipidomics**

We conducted LC-MS/MS analysis as described previously [2, 3]. Lipid metabolites were extracted by solid-phase extraction using Monospin C18-AX cartridges (GL Science, Shinjuku, Tokyo, Japan) in the presence of deuterated internal standard: 1 ng of AA-d8, 15-hydroxyeicosatetraenoic acid (HETE)-d8, leukotriene B4 (LTB4)-d4, LTD4-d5, prostaglandin E2 (PGE2)-d4, PGB2-d4, and 9-iso-PGF2α-d4. For LC-MS/MS analysis, a triple-quadrupole linear ion-trap mass spectrometer (5500QTRAP; Sciex,
Framingham, MA, USA) equipped with an ACQUITY UPLC BEH C18 column (1.0 × 150 mm, 1.7-µm particle size; Waters, Milford, MA, USA) was used. MS/MS analyses were conducted in negative ion mode, and lipid metabolites were identified and quantified by multiple reaction monitoring. Calibration curves between 1 and 1000 pg and the LC retention times for each compound were established with synthetic standards.

Statistical analysis

Statistical significance was evaluated using an unpaired t test. For multiplex comparisons, the one-way analysis of variance test with Tukey’s test was used. P < 0.05 was considered statistically significant. Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software).

Supplementary References

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[3] Isobe Y, Itagaki M, Ito Y, Naoe S, Kojima K, Ikeguchi M, Arita M. Comprehensive analysis of the mouse cytochrome P450 family responsible for omega-3 epoxidation of eicosapentaenoic acid. Sci Rep. 2018; 8(1):7954-4; doi: 10.1038/s41598-018-26325-4 [doi].
### Supplementary Table

| Sample Name       | Sham WT (n = 6) | Sham KO (n = 6) | Nx WT (n = 4) | Nx KO (n = 4) | p value (Nx WT vs Nx KO) |
|-------------------|----------------|----------------|--------------|--------------|--------------------------|
|                   | Ave [pg]       | SE             | Ave [pg]     | SE           |                          |
| PGE2              | 29.0           | 12.8           | 68.8         | 46.0         | 19.3                     | 13.3 | 0.9575       |
| PGD2              | 10.0           | 1.5            | 16.5         | 5.9          | 53.4                     | 14.5 | 0.0093       |
| 15-keto-PGE2      | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 15-deoxy-PGJ2     | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| PGF2a             | 2.4            | 0.9            | 5.8          | 0.4          | 1.6                      | 0.9  | 0.1552       |
| 6-keto-PGF1a      | 0.0            | 0.0            | 4.8          | 4.8          | 134.4                    | 26.1 | 0.9518       |
| TXB2              | 0.0            | 0.0            | 2.9          | 2.9          | 16.5                     | 6.1  | 0.1267       |
| 12-HHTxE          | 12.3           | 2.2            | 17.5         | 2.9          | 38.4                     | 7.9  | 0.127        |
| LTB4              | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| LTB4-20OH         | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| LTD4              | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| HxA3              | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| HxB3              | 20.1           | 20.1           | 0.0          | 0.0          | 20.4                     | 20.4 | 0.815        |
| LXA4              | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| LXB4              | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 5-HETE            | 114.1          | 17.1           | 198.4        | 13.7         | 91.5                     | 14.4 | 0.9938       |
| 5,6-EET           | 32.9           | 5.3            | 48.2         | 5.9          | 35.1                     | 8.9  | 0.4308       |
| 5,6-DHT           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 8-HETE            | 46.2           | 2.3            | 68.8         | 4.8          | 39.6                     | 3.3  | 0.6138       |
| 9-HETE            | 17.9           | 17.9           | 110.7        | 35.5         | 0.0                      | 0.0  | >0.9999      |
| 8,9-EET           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 8,9-DHT           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 11-HETE           | 57.1           | 6.6            | 99.3         | 9.9          | 65.5                     | 3.6  | >0.9999      |
| 12-HETE           | 141.9          | 20.8           | 213.5        | 39.2         | 175.8                    | 9.8  | 0.9996       |
| 11,12-EET         | 3.2            | 1.1            | 8.1          | 0.6          | 2.9                      | 1.7  | 0.0863       |
| 11,12-DHT         | 0.0            | 0.0            | 3.5          | 2.4          | 2.5                      | 2.5  | 0.998        |
| 15-HETE           | 125.6          | 18.2           | 241.7        | 25.8         | 177.2                    | 18.8 | 0.2103       |
| 14,15-EET         | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 14,15-DHT         | 3.6            | 1.7            | 9.9          | 1.5          | 8.3                      | 1.3  | 0.8897       |
| 16-HETE           | 2.2            | 1.4            | 9.4          | 3.2          | 7.0                      | 1.6  | 0.2338       |
| 17-HETE           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 18-HETE           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 19-HETE           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 20-HETE           | 0.0            | 0.0            | 0.0          | 0.0          | 0.0                      | 0.0  | -            |
| 5-oxo-ETE         | 27.4           | 9.2            | 28.2         | 4.9          | 13.1                     | 5.9  | 0.9987       |
| Compound          | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | -  |
|-------------------|-----|-----|-----|-----|-----|-----|-----|----|
| 12-oxo-ETE        | 25.4| 8.6 | 36.2| 4.7 | 11.0| 6.1 | 10.1| 3.9|0.9997|
| 15-oxo-ETE        | 4.9 | 4.9 | 7.6 | 7.6 | 0.0 | 0.0 | 0.0 | 0.0|>0.9999|
| 5,15-diHETE       | 0.0 | 0.0 | 28.8| 9.9 | 15.5| 15.5| 15.4| 15.4|>0.9999|
| PGE3              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| PGD3              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| PGF3a             | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 6-keto-PGF1a-17delta| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| TXB3              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| LTB5              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| LXA5              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| RvE1              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| RvE2              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 5-HEPE            | 99.3| 11.7| 124.8|12.9| 120.0|10.8| 91.2|20.8|0.5754|
| 5,6-diHETE        | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 8-HEPE            | 26.0| 6.8 | 55.5| 8.6 | 57.8| 4.1 | 35.7| 8.6|0.2974|
| 9-HEPE            | 59.4| 21.0|119.0|10.9|109.0|20.3| 49.2|13.8|0.1706|
| 8,9-EEPE          | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 8,9-diHEPE        | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 11-HEPE           | 60.4| 7.6 | 95.0|12.2|101.6|19.7| 51.7|11.8|0.0946|
| 12-HEPE           | 230.3|44.1|399.0|82.8|263.8|34.1|274.7|104.9|0.9997|
| 11,12-EEPE        | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 11,12-diHETE      | 0.0 | 0.0 | 0.0 | 0.0 | 9.4 | 9.4 | 11.7|11.7|0.9947|
| 15-HEPE           | 78.8| 8.7 | 99.4| 8.1 | 219.2|26.7| 89.5|25.9|0.0006|
| 14,15-EEPE        | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 14,15-diHETE      | 60.0|15.6| 50.9|23.4| 97.0|12.9| 96.5|30.1| -   |
| 18-HEPE           | 136.9|13.3|215.7|17.6|260.8|55.7|118.3|21.7|0.0186|
| 17,18-EEPE        | 0.0 | 0.0 | 0.0 | 0.0 | 28.0|28.0| 0.0 | 0.0|0.3891|
| 17,18-diHETE      | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 19-HEPE           | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 20-HEPE           | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 8,18-dHEPE        | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 11,18-diHEPE-2    | 5.3 | 2.4 | 6.9 | 4.6 | 13.1| 4.4 | 0.0 | 0.0|0.1368|
| 12,18-diHEPE      | 0.0 | 0.0 | 0.0 | 0.0 | 54.5|23.0| 0.0 | 0.0|0.0067|
| 17,18-diHEPE-RS   | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 12-h-17,18-EEPE   | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 14,15-17,18-diEEPE| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| RvD1              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| RvD2              | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0| -   |
| 4-HDoHE | 396.8 | 55.8 | 646.8 | 74.6 | 417.3 | 70.2 | 360.5 | 91.7 | 0.959 |
|---------|-------|------|-------|------|-------|------|------|------|------|
| 7-HDoHE | 75.1  | 4.0  | 108.9 | 10.7 | 107.7 | 8.9  | 69.0  | 13.8 | 0.0829 |
| 8-HDoHE | 337.7 | 43.4 | 585.3 | 74.9 | 335.7 | 42.2 | 284.4 | 47.0 | 0.9439 |
| 7,8-EpDPE | 31.4 | 7.3 | 38.7 | 7.5 | 47.6 | 7.4 | 67.4 | 16.9 | 0.5772 |
| 7,8-dHDoPE | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - |
| 10-HDoHE | 128.8 | 16.5 | 256.9 | 30.7 | 288.1 | 54.7 | 126.7 | 25.4 | 0.0246 |
| 11-HDoHE | 245.9 | 43.2 | 488.0 | 74.5 | 678.4 | 146.6 | 305.5 | 57.8 | 0.0443 |
| 10,11-EpDPE | 25.7 | 4.5 | 32.9 | 4.1 | 21.0 | 3.3 | 40.5 | 7.3 | 0.092 |
| 10,11-dHDoPE | 2.6 | 2.6 | 6.1 | 2.3 | 4.4 | 4.4 | 11.7 | 9.6 | 0.7582 |
| 13-HDoHE | 140.8 | 17.4 | 255.2 | 26.8 | 388.1 | 104.6 | 162.8 | 31.2 | 0.0341 |
| 14-HDoHE | 289.5 | 25.1 | 368.6 | 26.2 | 673.2 | 128.3 | 328.0 | 57.6 | 0.0092 |
| 13,14-EpDPE | 13.4 | 1.9 | 19.5 | 1.8 | 14.8 | 3.6 | 16.8 | 3.6 | 0.9631 |
| 13,14-dHDoPE | 21.2 | 4.0 | 23.8 | 4.7 | 19.9 | 5.9 | 24.9 | 13.8 | 0.9663 |
| 16-HDoHE | 232.1 | 25.1 | 369.1 | 38.3 | 471.7 | 106.7 | 227.0 | 53.4 | 0.0497 |
| 17-HDoHE | 468.5 | 82.0 | 513.1 | 59.0 | 1253.0 | 218.2 | 350.6 | 88.7 | 0.0005 |
| 16,17-EpDPE | 7.3 | 3.3 | 15.7 | 3.5 | 7.4 | 4.7 | 5.0 | 5.0 | 0.9789 |
| 16,17-dHDoPE | 29.9 | 1.6 | 32.4 | 6.8 | 29.9 | 5.7 | 27.5 | 4.8 | 0.9912 |
| 20-HDoHE | 398.9 | 59.6 | 700.2 | 78.1 | 766.0 | 206.3 | 388.5 | 75.4 | 0.1414 |
| 19,20-EpDPE | 220.7 | 12.4 | 203.5 | 20.7 | 319.2 | 50.7 | 218.1 | 39.2 | 0.1621 |
| 19,20-dHDoPE | 93.4 | 7.9 | 75.8 | 12.3 | 102.4 | 10.8 | 105.8 | 24.3 | 0.9986 |
| 21-HDoHE | 116.1 | 15.0 | 118.7 | 12.7 | 214.9 | 50.9 | 124.7 | 18.9 | 0.1271 |
| 22-HDoHE | 0.0 | 0.0 | 8.2 | 5.2 | 15.9 | 9.2 | 12.7 | 7.4 | 0.9825 |
| 17-axo-DoHE | 27.8 | 12.7 | 56.2 | 16.7 | 99.5 | 25.4 | 67.6 | 22.6 | 0.6958 |
| 4,14-dHDoHE | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - |
| 7,14-axHDoHE | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - |
| 7,17-dHDoHE | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | - |
| 10,17-dHDoHE | 0.0 | 0.0 | 5.6 | 5.6 | 21.9 | 8.0 | 0.0 | 0.0 | 0.0381 |
| 14,20-dHDoHE | 0.0 | 0.0 | 31.1 | 21.5 | 104.6 | 28.1 | 0.0 | 0.0 | 0.0067 |
| 16:0 Palmitic Acid | 91902.6 | 10170.4 | 82738.2 | 5263.1 | 191861.6 | 22821.2 | 143310.6 | 27204.8 | 0.2328 |
| 16:1 Palmitoleic Acid | 15658.9 | 3956.6 | 9630.7 | 1623.5 | 81329.7 | 17316.3 | 48814.0 | 14128.6 | 0.1459 |
| 18:0 Stearic Acid | 45670.5 | 4471.5 | 48051.3 | 3748.8 | 58167.9 | 8210.8 | 55004.4 | 6115.5 | 0.9814 |
| 18:1 Oleic Acid(9) | 62253.5 | 9160.4 | 51236.5 | 4697.2 | 14910.4 | 18033.3 | 118453.8 | 18809.9 | 0.3889 |
| 18:2 Linoleic Acid | 206766.9 | 25926.2 | 177144.1 | 15532.9 | 58290.5 | 71210.7 | 415077.8 | 84585.1 | 0.1346 |
| 18:3 a-Linolenic Acid(3) | 17294.0 | 3733.9 | 14980.1 | 1876.3 | 85228.7 | 10241.5 | 50273.1 | 16420.4 | 0.0571 |
| 18:3 g-Linolenic Acid(n6) | 1191.7 | 133.2 | 1089.2 | 100.4 | 2957.9 | 282.8 | 1996.5 | 589.1 | 0.1579 |
| 18:4 Stearidonic Acid | 1169.9 | 178.1 | 1211.4 | 142.2 | 5502.3 | 738.9 | 3694.6 | 1387.2 | 0.2957 |
| 20:3 DGLA(n6) | 9794.6 | 349.6 | 8797.8 | 791.8 | 22167.8 | 3136.0 | 13040.0 | 2866.7 | 0.0195 |
| 20:3 Mead Acid(9) | 222.7 | 24.9 | 224.1 | 32.7 | 359.4 | 68.6 | 314.7 | 29.7 | 0.8806 |
| 20:4 ETA(n3) | 1098.3 | 134.0 | 1090.4 | 78.2 | 4297.5 | 752.6 | 2587.0 | 812.5 | 0.1049 |
Supplementary Table 1

List of all the lipid metabolites in sham and 5/6 Nx kidneys which were analyzed with the mediator lipidomics. By mediator lipidomics, the above fatty acid metabolites were detected in the kidney tissue (30 mg). The P values in the table were obtained by comparing Alox15+/+ and Alox15−/− mice under 5/6 Nx condition. The number of samples is as follows: sham (WT), n = 6, sham (KO), n = 6, Nx (WT), n = 4, Nx (KO), n = 4. One-way analysis of variance was followed by Tukey’s multiple comparisons test.

| Metabolite               | sham (WT)  | sham (KO) | Nx (WT)  | Nx (KO)  | P value |
|--------------------------|------------|-----------|----------|----------|---------|
| 20:4 Arachidonic Acid(n6)| 47987.9    | 49800.5   | 4022.6   | 45471.4  | 0.9837  |
| 20:5 EPA                 | 30513.0    | 32268.5   | 2776.5   | 33619.7  | 0.7669  |
| 22:4 Adrenic Acid        | 1777.1     | 2004.2    | 237.4    | 3741.6   | 0.0696  |
| 22:5 DPA(n3)             | 24774.2    | 25339.4   | 2371.4   | 31422.8  | 0.1596  |
| 22:5 Osbond Acid(n6)     | 1547.1     | 1380.3    | 209.9    | 3673.0   | 0.1312  |
| 22:6 DHA                 | 222989.5   | 219463.9  | 17716.7  | 207464.4 | 0.5304  |

By mediator lipidomics, the above fatty acid metabolites were detected in the kidney tissue (30 mg). The P values in the table were obtained by comparing Alox15+/+ and Alox15−/− mice under 5/6 Nx condition. The number of samples is as follows: sham (WT), n = 6, sham (KO), n = 6, Nx (WT), n = 4, Nx (KO), n = 4. One-way analysis of variance was followed by Tukey’s multiple comparisons test.