

Supplementary Information for

The PDK1-Foxo1 signaling in adipocytes controls systemic insulin sensitivity through the 5-lipoxygenase- leukotriene B4 axis

Tetsuya Hosooka,^{1,a} Yusei Hosokawa,^{1,a} Kaku Matsugi,¹ Masakazu Shinohara,^{2,3} Yoko Senga,¹ Yoshikazu Tamori,^{1,4} Chikako Aoki,¹ Sho Matsui,⁵ Tsutomu Sasaki,⁵ Tadahiro Kitamura,⁵ Masashi Kuroda,⁶ Hiroshi Sakaue,⁶ Kazuhiro Nomura,¹ Kei Yoshino,¹ Yuko Nabatame,¹ Yoshito Itoh,⁷ Kanji Yamaguchi,⁷ Yoshitake Hayashi,⁸ Jun Nakae,⁹ Domenico Accilli,¹⁰ Takehiko Yokomizo,¹¹ Susumu Seino,¹² Masato Kasuga¹³ & Wataru Ogawa¹

Corresponding author: Wataru Ogawa

Email: ogawa@med.kobe-u.ac.jp

This PDF file includes:

Figures S1 to S9

Tables S1 to S3

Fig. S1

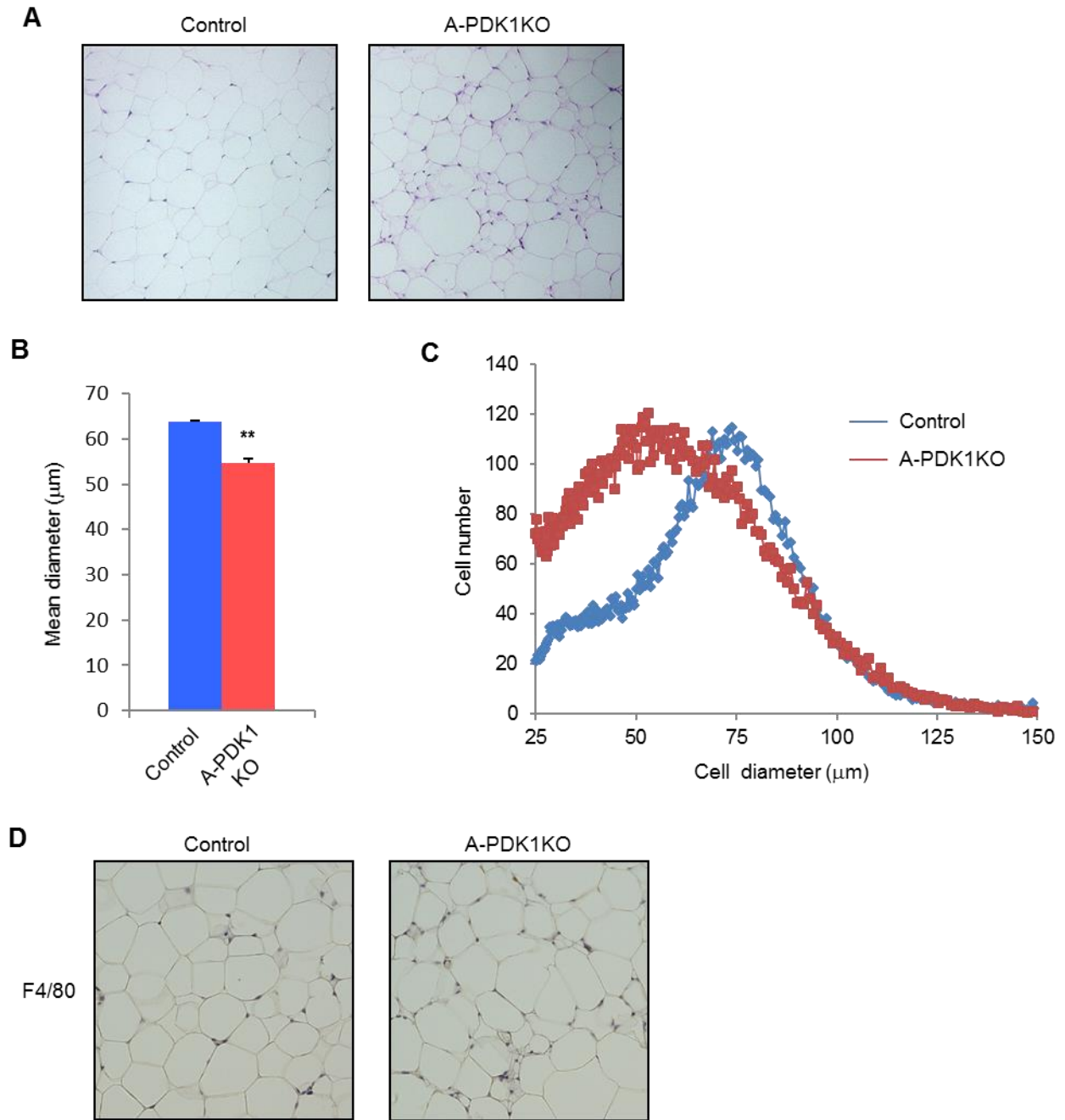


Fig. S1. Decreased adipocyte size in A-PDK1KO mice. (A) Hematoxylin-eosin staining of epididymal adipose tissue from control and A-PDK1KO mice. (B and C) Mean diameter of adipocytes (B) and distribution of adipocyte size (C) for epididymal adipose tissue from 20-week-old control or A-PDK1KO mice ($n = 4$). Data in (B) are means \pm s.e.m. ** $P < 0.01$ versus control (Student's t test). (D) F4/80 immunohistochemistry of epididymal adipose tissue from control and A-PDK1KO mice.

Fig. S2

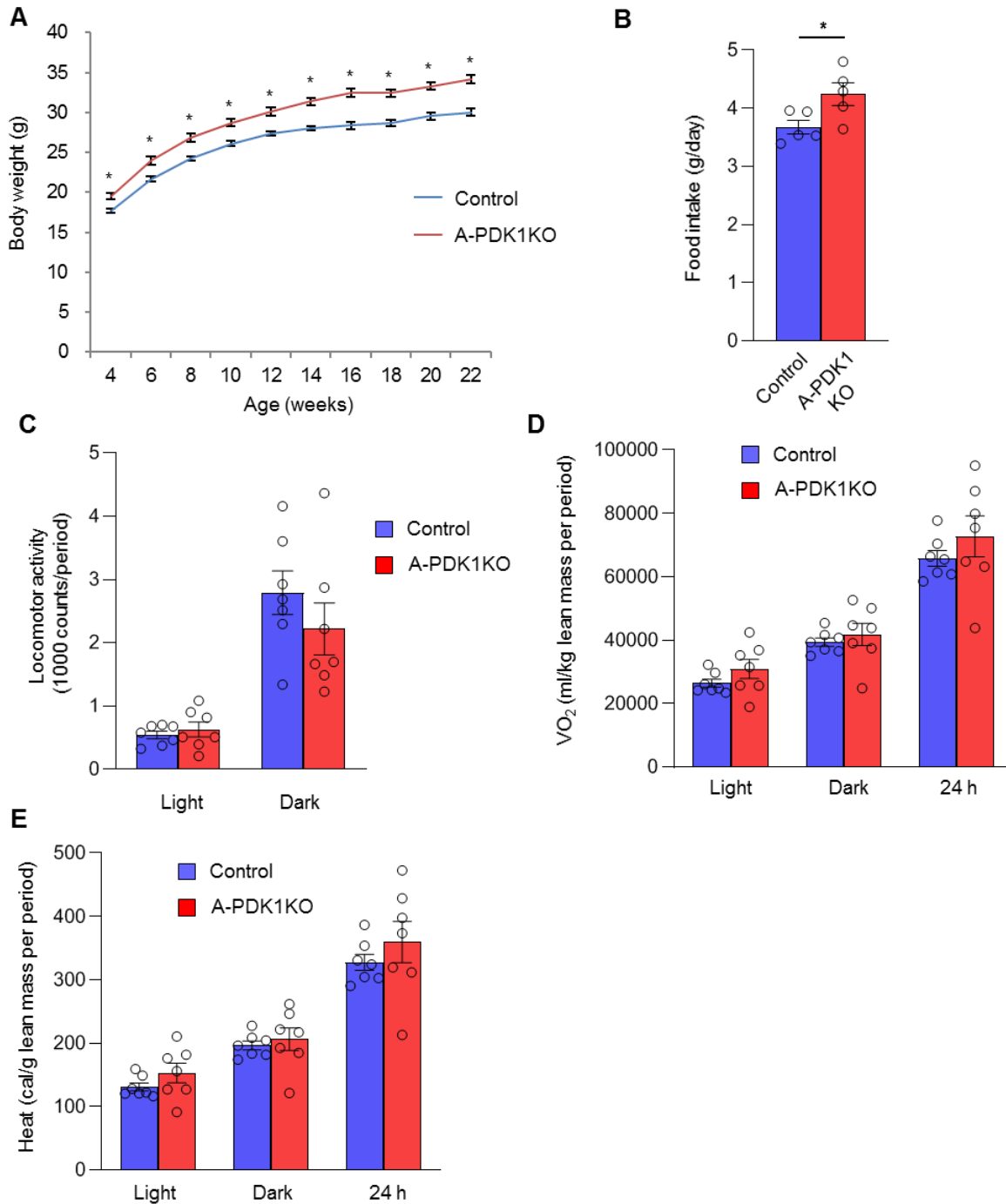


Fig. S2. Body weight, food intake, locomotor activity, and energy expenditure in A-PDK1KO mice. (A) Time course of body weight for control or A-PDK1KO mice ($n = 7$ to 10). (B) Food intake for 15-week-old control or A-PDK1KO mice ($n = 5$). (C-E) Locomotor activity (C), oxygen consumption (VO_2) (D), and heat production (E) during light, dark, and 24-h periods for 14-week-old control or A-PDK1KO mice ($n = 7$). All data are means \pm s.e.m. * $P < 0.05$ versus corresponding control value (Student's t test).

Fig. S3

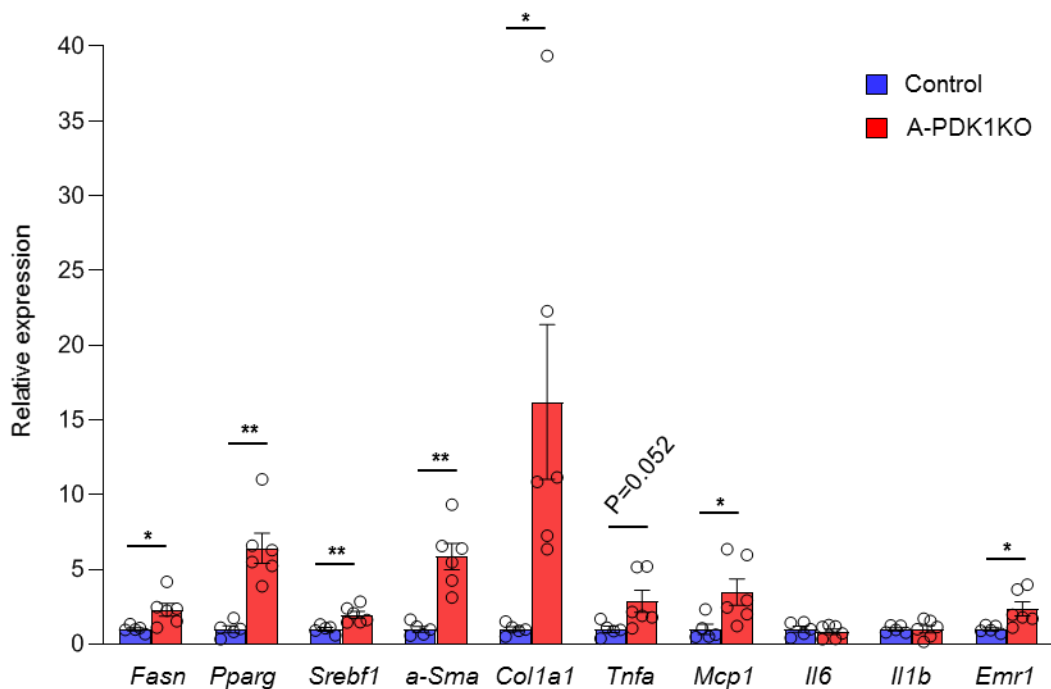


Fig. S3. Gene expression in the liver of A-PDK1KO mice. The abundance of mRNAs for genes related to lipogenesis, fibrosis, or inflammation in the liver of 37-week-old control or A-PDK1KO mice ($n = 5-6$) was determined by Real-time PCR analysis. Data are means \pm s.e.m. * $P < 0.05$. ** $P < 0.01$ versus corresponding control value (Student's t test).

Fig. S4

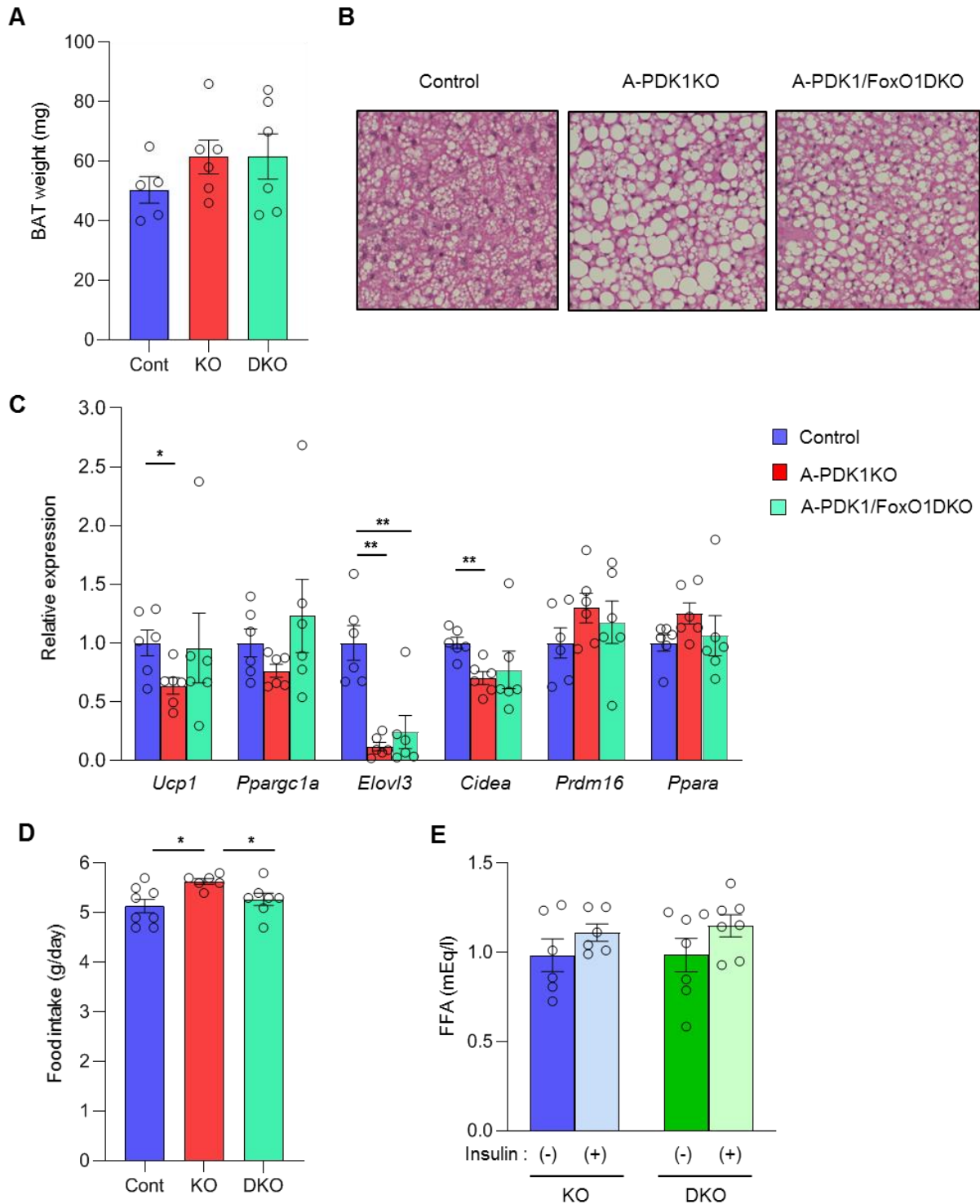


Fig. S4. BAT weight and histology, food intake, and lipolysis in A-PDK1KO and A-PDK1/FoxO1DKO mice. (A) BAT weight in control, A-PDK1KO, and A-PDK1/FoxO1DKO mice ($n = 5$ or 6). (B) Hematoxylin-eosin staining of BAT from control, A-PDK1KO, and A-PDK1/FoxO1DKO mice. (C) Gene expression in BAT from control, A-PDK1KO, and A-PDK1/FoxO1DKO mice ($n = 6$). (D) Food intake in control, A-PDK1KO, and A-

PDK1/FoxO1DKO mice ($n = 6$ to 8) (E) Plasma FFA concentration in A-PDK1KO and A-PDK1/FoxO1DKO mice ($n = 6$ or 7) measured 1 h after intraperitoneal injection of insulin (1 U/kg) or vehicle. All data are means \pm s.e.m. * $P < 0.05$. ** $P < 0.01$ versus corresponding control value (Student's t test).

Fig. S5

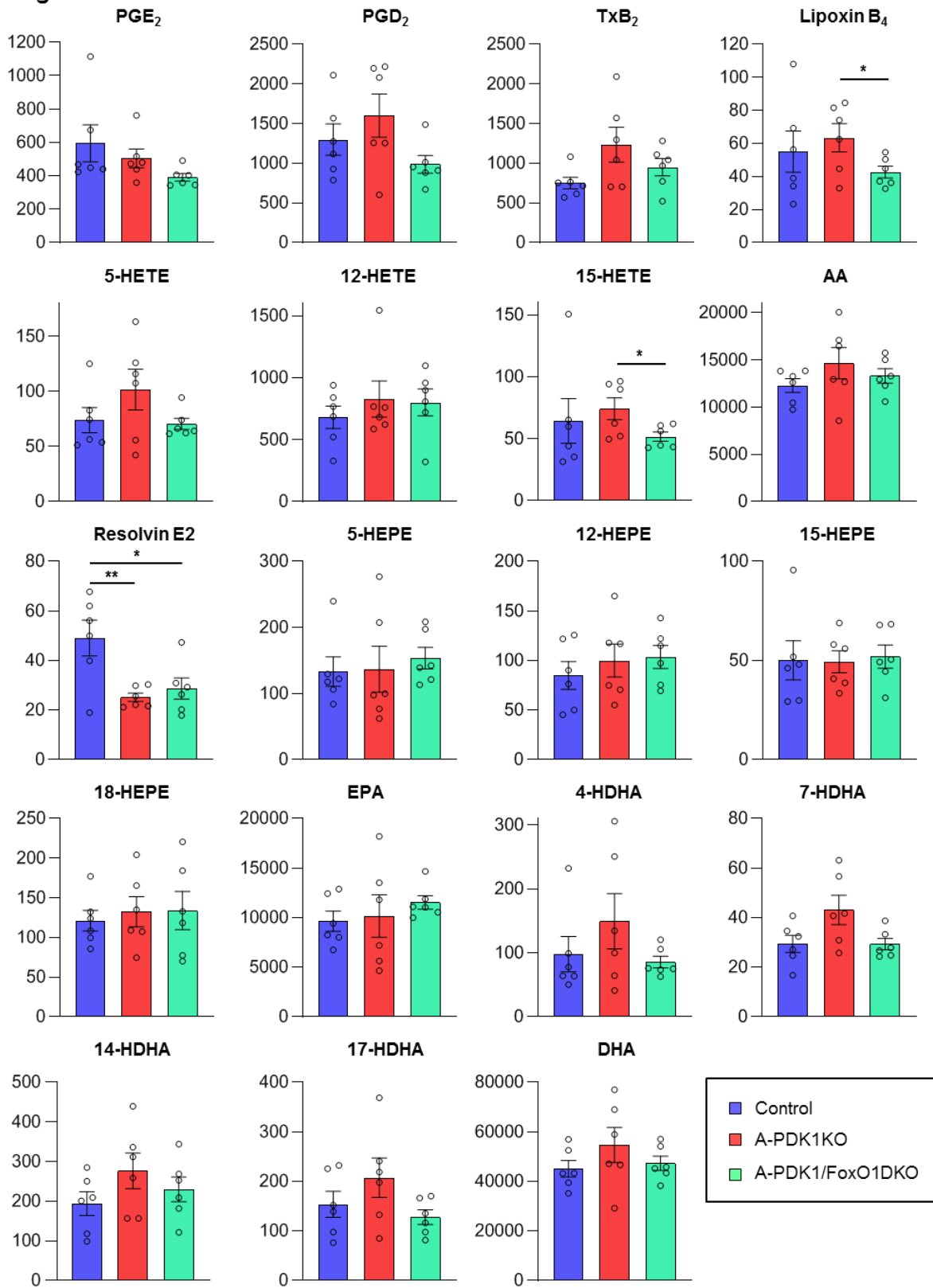


Fig. S5. Plasma concentrations of various lipid mediators in A-PDK1KO and A-PDK1/FoxO1DKO mice. The plasma concentrations of lipid mediators in 13-week-old control, A-PDK1KO, or A-PDK1/FoxO1DKO mice ($n = 6$) were measured by LC-MS/MS analysis. All data are presented as picograms per milliliter and are means \pm s.e.m. * $P < 0.05$. ** $P < 0.01$ versus corresponding control value (Student's t test). PGE₂, prostaglandin E₂; PGD₂, prostaglandin D₂; TxB₂, thromboxane B₂; 5-HETE, 5-hydroxyeicosatetraenoic acid; 12-HETE, 12-hydroxyeicosatetraenoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; AA, arachidonic acid; 5-HEPE, 5-hydroxyeicosapentaenoic acid; 12-HEPE, 12-hydroxyeicosapentaenoic acid; 15-HEPE, 15-hydroxyeicosapentaenoic acid; 18-HEPE, 18-hydroxyeicosapentaenoic acid; EPA, eicosapentaenoic acid; 4-HDHA, 4-hydroxydocosahexaenoic acid; 7-HDHA, 7-hydroxydocosahexaenoic acid; 14-HDHA, 14-hydroxydocosahexaenoic acid; 17-HDHA, 17-hydroxydocosahexaenoic acid; DHA, docosahexaenoic acid.

Fig. S6

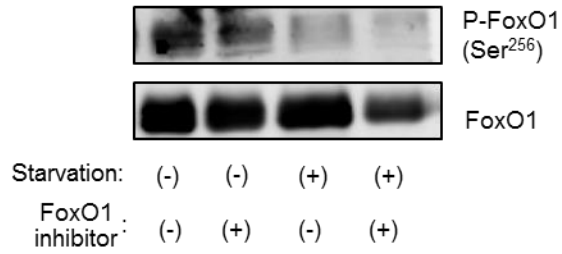


Fig. S6. Serum deprivation–induced down-regulation of FoxO1 phosphorylation in SV cells. SV cells isolated from C57BL/6 mice were exposed to AS1842856 (10 μ M) during serum deprivation for 24 h and were then subjected to immunoblot analysis of total and Ser²⁵⁶-phosphorylated forms of FoxO1.

Fig. S7

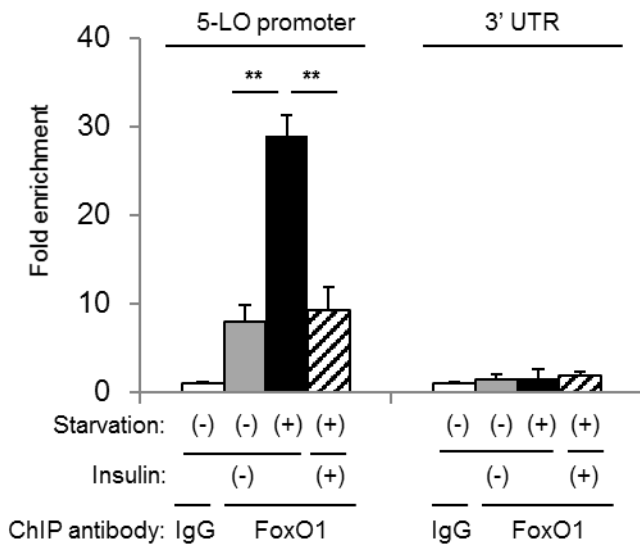


Fig. S7. FoxO1 binds to the 5-LO promoter in 3T3L1 adipocytes. ChIP analysis of FoxO1 binding to the 5-LO promoter in 3T3L1 adipocytes treated with (or without) insulin for 1 hour after serum starvation for 12 hours ($n = 3$). Data are representative of three independent experiments. Data are means \pm s.e.m. $**P < 0.01$ (Student's *t* test).

Fig. S8

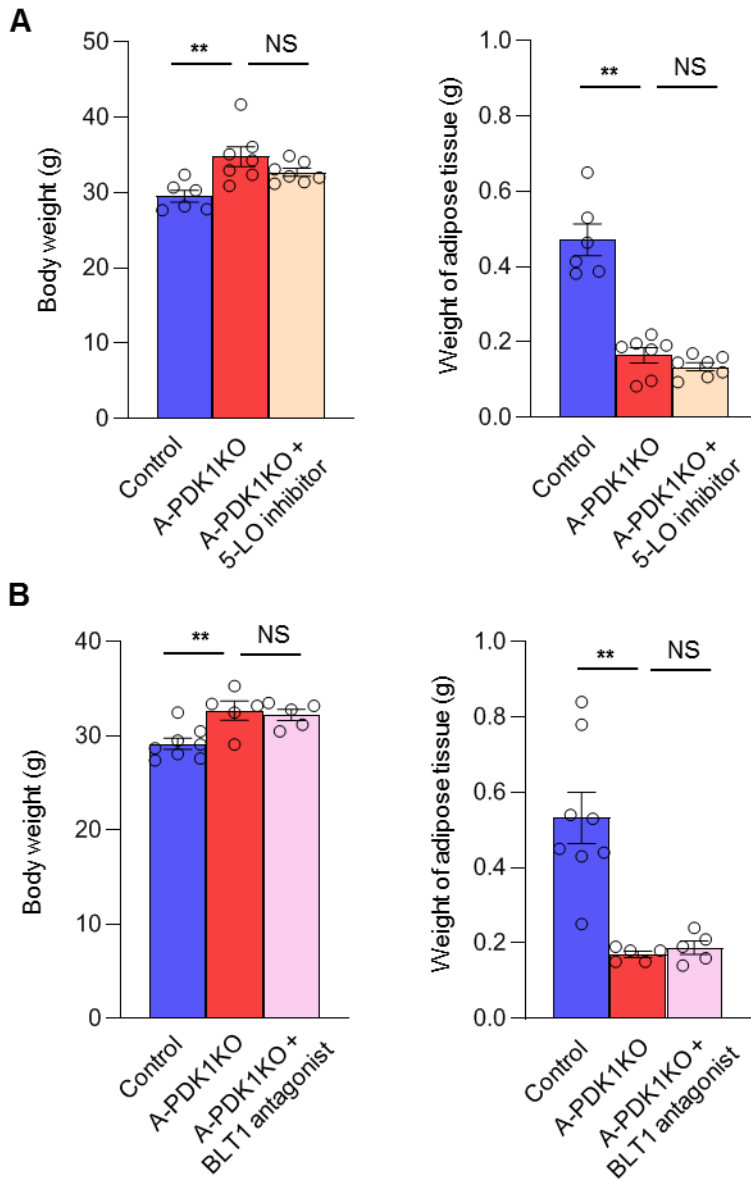


Fig. S8. Body and adipose tissue weight in A-PDK1KO mice treated with 5-LO inhibitor zileuton or BLT1 antagonist CP105696. (A) Body and adipose tissue weight in control or A-PDK1KO mice ($n = 6$ or 7) treated (or not) with zileuton for 12 weeks. (B) Body and adipose tissue weight in control or A-PDK1KO mice ($n = 5$ to 8) treated (or not) with CP105696 for 12 weeks. All data are means \pm s.e.m. ** $P < 0.01$ (Student's t test).

Fig. S9

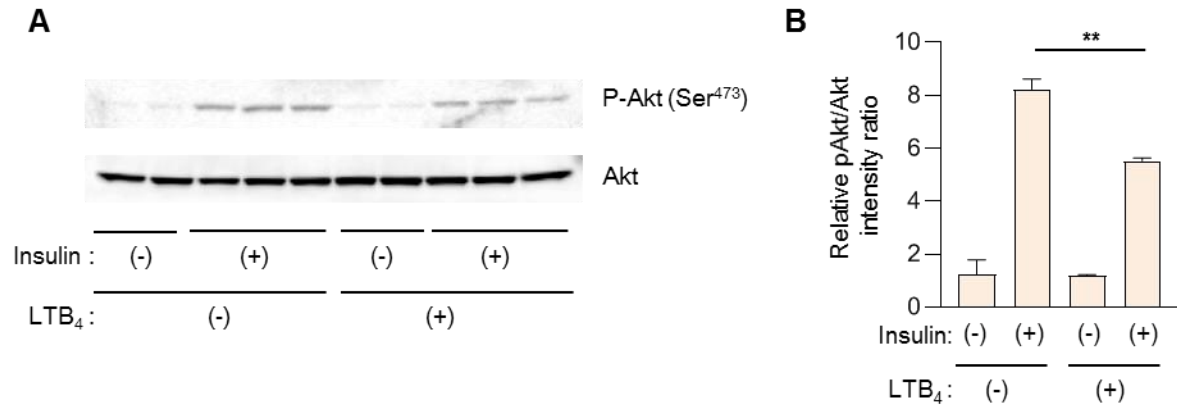


Fig. S9. LTB₄ suppresses insulin-dependent Akt phosphorylation in mouse primary hepatocytes. (A) Representative immunoblot of total and Ser473-phosphorylated forms of Akt in mouse primary hepatocytes stimulated with (or without) insulin after LTB₄ (100nM) or vehicle treatment for 1 hour. (B) Quantifications of experiments shown in panel (A). Data are representative of three independent experiments. Data are means \pm s.e.m. **P < 0.01 (Student's t test).

Table S1. List of genes whose expression in epididymal adipose tissue was increased ($\log_2(\text{KO/control ratio}) > 0.5$) in A-PDK1KO mice and normalized ($\log_2(\text{DKO/control ratio}) < 0.2$) in A-PDK1/FoxO1DKO mice among genes with an expression signal of >30 in control mice. Data for the 5-LO gene (*Alox5*) are shown in red.

| Gene symbol | Gene accession | Probe set ID | Control signal | KO signal | $\log_2(\text{KO/control})$ | DKO signal | $\log_2(\text{DKO/control})$ |
|---------------|--------------------|--------------|----------------|-----------|-----------------------------|------------|------------------------------|
| Rnu1b1 | NR_004412 | 17219206 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Ankrd23 | NM_153502 | 17222193 | 55.5 | 78.5 | 0.5 | 60.4 | 0.12 |
| Elf3 | NM_001163131 | 17227266 | 30.2 | 49.6 | 0.72 | 31.5 | 0.06 |
| Prg4 | NM_021400 | 17227892 | 88.5 | 185.2 | 1.07 | 85.9 | -0.04 |
| Akap12 | NM_031185 | 17231423 | 305.5 | 481.5 | 0.66 | 318.1 | 0.06 |
| Vnn1 | NM_011704 | 17232162 | 61.7 | 142.9 | 1.21 | 37.3 | -0.72 |
| Elane | NM_015779 | 17235011 | 50.1 | 72.1 | 0.52 | 54.9 | 0.13 |
| 2210404O07Rik | ENSMUST00000105322 | 17235730 | 30.2 | 53.6 | 0.83 | 29.1 | -0.05 |
| Eid3 | NM_025499 | 17235937 | 30.8 | 63.8 | 1.05 | 33.2 | 0.11 |
| Rdh16 | NM_009040 | 17238172 | 57.3 | 90.3 | 0.66 | 48.2 | -0.25 |
| Syne1 | ENSMUST00000056571 | 17238904 | 38.9 | 60.1 | 0.63 | 44.2 | 0.18 |
| Syne1 | ENSMUST00000056571 | 17238930 | 72.5 | 111.1 | 0.62 | 83.2 | 0.2 |
| Col18a1 | NM_009929 | 17242232 | 83.4 | 125.1 | 0.59 | 91.8 | 0.14 |
| Zip781 | NM_199062 | 17243460 | 76.1 | 112.1 | 0.56 | 55.8 | -0.45 |
| 3230401D17Rik | ENSMUST00000104954 | 17249700 | 40 | 57.3 | 0.52 | 43.9 | 0.14 |
| Lrrc48 | ENSMUST00000108723 | 17250344 | 32.7 | 60.5 | 0.89 | 35.6 | 0.12 |
| Adap2 | ENSMUST00000021050 | 17253885 | 406.2 | 742.4 | 0.87 | 354.7 | -0.2 |
| Gngt2 | ENSMUST00000036088 | 17255466 | 30.2 | 44.7 | 0.57 | 31 | 0.04 |
| Spns2 | NM_153060 | 17265672 | 174.5 | 248.4 | 0.51 | 189 | 0.11 |
| Recql5 | NM_130454 | 17272047 | 40.7 | 68.3 | 0.75 | 44 | 0.11 |
| Mia2 | NM_177321 | 17275718 | 42.7 | 83.8 | 0.97 | 47.5 | 0.16 |
| Irf4 | ENSMUST00000021784 | 17286295 | 82.3 | 140.5 | 0.77 | 68.9 | -0.26 |
| Gcnt2 | NM_008105 | 17286707 | 121.3 | 200.4 | 0.72 | 127 | 0.07 |
| Ankrd32 | NM_134071 | 17294518 | 57 | 83.1 | 0.54 | 65.2 | 0.19 |
| Selk | NM_019979 | 17298131 | 53.4 | 92.5 | 0.79 | 52 | -0.04 |
| Anxa8 | NM_013473 | 17298775 | 66.8 | 101.7 | 0.61 | 56.6 | -0.24 |
| AY358078 | AY358078 | 17299627 | 54.1 | 79.6 | 0.56 | 44 | -0.3 |
| 1700047G03Rik | NR_040447 | 17310400 | 62.1 | 104.4 | 0.75 | 43.3 | -0.52 |
| Dnajc22 | ENSMUST00000061295 | 17314729 | 33.1 | 46.9 | 0.5 | 34.2 | 0.05 |
| Krt18 | NM_010664 | 17315245 | 77.8 | 132 | 0.76 | 87.9 | 0.18 |
| Egflam | NM_178748 | 17315763 | 54.2 | 79 | 0.54 | 51.8 | -0.07 |
| Kcnj15 | ENSMUST00000113862 | 17327450 | 54.6 | 78.4 | 0.52 | 46.2 | -0.24 |
| Prm2 | NM_008933 | 17328113 | 143.2 | 645.2 | 2.17 | 99.2 | -0.53 |
| Prm1 | ENSMUST00000023144 | 17328118 | 35.6 | 79.8 | 1.16 | 31.8 | -0.17 |
| Ube2v2 | NM_023585 | 17328425 | 46.1 | 72.4 | 0.65 | 52.8 | 0.2 |
| Apod | NM_007470 | 17329759 | 195.4 | 276.4 | 0.5 | 183.8 | -0.09 |
| Gm609 | NM_001005854 | 17330636 | 36.7 | 63 | 0.78 | 40.4 | 0.14 |
| Pvrl3 | NM_021495 | 17330773 | 43 | 61.2 | 0.51 | 47 | 0.13 |
| Tmem45a | NM_019631 | 17331078 | 31.8 | 46.3 | 0.54 | 33.3 | 0.06 |
| Cdkn1a | NM_007669 | 17335467 | 287.5 | 413.6 | 0.52 | 330.2 | 0.2 |
| Gm16519 | ENSMUST00000092011 | 17339390 | 70.8 | 122.1 | 0.79 | 75.3 | 0.09 |
| Vit | NM_028813 | 17339824 | 66.6 | 98 | 0.56 | 63.7 | -0.06 |
| Thbs2 | NM_011581 | 17341080 | 178.3 | 477.7 | 1.42 | 86.1 | -1.05 |
| Tcp11 | NM_013687 | 17342810 | 30.4 | 51 | 0.74 | 29.1 | -0.07 |
| Plk7 | NM_175168 | 17345475 | 33.4 | 49.3 | 0.56 | 37.6 | 0.17 |
| Tgif1 | NM_001164075 | 17346936 | 42 | 63.6 | 0.6 | 45.1 | 0.1 |
| Ankrd29 | NM_001190371 | 17352763 | 105.4 | 156.9 | 0.57 | 102.9 | -0.04 |
| Ak3 | NM_021299 | 17358498 | 35.4 | 51 | 0.53 | 35.8 | 0.01 |
| BC021614 | NM_144869 | 17361090 | 31.6 | 46.7 | 0.56 | 25.4 | -0.32 |
| Ms4a14 | XM_357051 | 17362941 | 32.9 | 54.1 | 0.72 | 22.2 | -0.56 |
| Sic16a12 | ENSMUST00000009522 | 17364139 | 107.4 | 180.2 | 0.75 | 101 | -0.09 |
| Gca | NM_145523 | 17371201 | 102.4 | 150.7 | 0.56 | 101.8 | -0.01 |
| Thbs1 | NM_011580 | 17374488 | 202.7 | 461.3 | 1.19 | 170.3 | -0.25 |
| Optn | ENSMUST00000114996 | 17381330 | 110.9 | 201.4 | 0.86 | 116.8 | 0.07 |
| 1700026L06Rik | NM_027283 | 17383423 | 37.2 | 54 | 0.54 | 38.4 | 0.04 |
| Fibin | NM_026271 | 17389157 | 34 | 53.4 | 0.65 | 36.3 | 0.09 |
| Rnu1b1 | NR_004412 | 17400568 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Rnu1b1 | NR_004412 | 17400579 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Rnu1b1 | NR_004412 | 17400588 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Rnu1b1 | NR_004412 | 17400590 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |

Table S1-continued.

| Gene symbol | Gene accession | Probe set ID | Control signal | KO signal | Log ₂ (KO/control) | DKO signal | Log ₂ (DKO/control) |
|---------------|--------------------|-----------------|----------------|-------------|-------------------------------|-------------|--------------------------------|
| Lef1 | ENSMUST00000029611 | 17402662 | 36.9 | 59 | 0.68 | 35.9 | -0.04 |
| Cetn4 | NM_145825 | 17404896 | 35 | 56.1 | 0.68 | 33.3 | -0.07 |
| Il6ra | NM_010559 | 17407138 | 392.1 | 619.4 | 0.66 | 370.5 | -0.08 |
| Rnu1b1 | NR_004412 | 17408038 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Rnu1b1 | NR_004412 | 17408063 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Rnu1b1 | NR_004412 | 17408065 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Synpo2 | NM_080451 | 17410031 | 74.1 | 114.1 | 0.62 | 83.3 | 0.17 |
| Acer2 | NM_139306 | 17415219 | 154.2 | 297.9 | 0.95 | 147.2 | -0.07 |
| Inadl | NM_172696 | 17415635 | 31.6 | 46.1 | 0.54 | 32.9 | 0.06 |
| Tic39a | NM_001145948 | 17416862 | 37.6 | 53.4 | 0.51 | 41.9 | 0.15 |
| Cldn19 | NM_153105 | 17417898 | 31.9 | 51.7 | 0.7 | 35.1 | 0.14 |
| Snord99 | NR_028537 | 17419411 | 501.8 | 756.9 | 0.59 | 550.9 | 0.13 |
| Hspg2 | NM_008305 | 17420171 | 755.4 | 1125.8 | 0.58 | 814.5 | 0.11 |
| Pla2g2d | NM_011109 | 17420530 | 154.8 | 332.7 | 1.1 | 167.7 | 0.12 |
| Plekhf2 | NM_175175 | 17423209 | 69.4 | 151.6 | 1.13 | 77.7 | 0.16 |
| Rnu1b1 | NR_004412 | 17425278 | 100.6 | 166.9 | 0.73 | 111.7 | 0.15 |
| Cyp2j13 | NM_145548 | 17427331 | 31.5 | 49.6 | 0.66 | 27.5 | -0.2 |
| Fam159a | NM_001099303 | 17428050 | 40 | 62.7 | 0.65 | 43.5 | 0.12 |
| Nasp | NM_016777 | 17428659 | 46 | 67.6 | 0.55 | 49.6 | 0.11 |
| Med18 | NM_026039 | 17430853 | 30.9 | 48.1 | 0.64 | 31.7 | 0.04 |
| Shroom3 | NM_015756 | 17439138 | 45.8 | 67.5 | 0.56 | 51.9 | 0.18 |
| Bmp3 | NM_173404 | 17439535 | 85.5 | 142.1 | 0.73 | 93.5 | 0.13 |
| Wdr66 | BC138176 | 17442237 | 41.9 | 63.8 | 0.61 | 47.9 | 0.19 |
| Fam20c | ENSMUST00000026972 | 17443901 | 96.4 | 156.5 | 0.7 | 98.6 | 0.03 |
| Gm9899 | NR_040427 | 17446752 | 34.6 | 50.8 | 0.55 | 37.9 | 0.13 |
| Pdk4 | NM_013743 | 17464654 | 287.5 | 490.8 | 0.77 | 307.5 | 0.1 |
| Gprn3 | ENSMUST00000051065 | 17467232 | 65.6 | 140.6 | 1.1 | 48.9 | -0.42 |
| Oxtr | ENSMUST00000165435 | 17469627 | 124.9 | 306.9 | 1.3 | 118.3 | -0.08 |
| Alox5 | NM_009662 | 17470031 | 46.8 | 69.2 | 0.56 | 52.1 | 0.15 |
| 4931406P16Rik | BC060233 | 17476690 | 35.1 | 51.9 | 0.57 | 38.4 | 0.13 |
| Stard10 | ENSMUST00000163799 | 17480813 | 214.7 | 327.2 | 0.61 | 198.3 | -0.11 |
| Acsn3 | NM_016870 | 17482310 | 160.9 | 308.3 | 0.94 | 103.9 | -0.63 |
| Olf310 | NM_001011520 | 17493116 | 48 | 68.9 | 0.52 | 46 | -0.06 |
| Defa3 | NM_007850 | 17499757 | 35.4 | 55.4 | 0.65 | 37.9 | 0.1 |
| Gse1 | NM_198671 | 17506249 | 63 | 90.9 | 0.53 | 70.9 | 0.17 |
| Ier2 | NM_010499 | 17511130 | 33.3 | 47.7 | 0.52 | 32.7 | -0.03 |
| Ccdc153 | NM_001081369 | 17516538 | 89.2 | 173.9 | 0.96 | 98 | 0.14 |
| Lctf | NM_145835 | 17518278 | 40 | 60.3 | 0.59 | 35.5 | -0.17 |
| Elmod1 | NM_177769 | 17527173 | 56.1 | 105.2 | 0.91 | 63 | 0.17 |
| Morf41 | NM_001039147 | 17529647 | 68.3 | 99 | 0.54 | 60.1 | -0.18 |
| Zbtb38 | ENSMUST00000152594 | 17529855 | 106.3 | 165.5 | 0.64 | 119.2 | 0.17 |
| Gm14484 | ENSMUST00000164729 | 17533307 | 34.7 | 51.5 | 0.57 | 39.1 | 0.17 |
| Fmr1 | NM_008031 | 17535208 | 600.3 | 929.1 | 0.63 | 528.6 | -0.18 |
| 1700011M02Rik | NM_001243285 | 17536945 | 57.7 | 83.5 | 0.53 | 51.3 | -0.17 |
| A730046J19Rik | NR_040271 | 17538350 | 33.4 | 50.3 | 0.59 | 34.6 | 0.05 |
| G530011O06Rik | NR_029457 | 17546212 | 43.8 | 88.1 | 1.01 | 48.3 | 0.14 |

Table S2. List of genes whose expression in epididymal adipose tissue was decreased ($\log_2(\text{KO}/\text{control ratio}) < -0.5$) in A-PDK1KO mice and normalized ($\log_2(\text{DKO}/\text{control ratio}) > -0.2$) in A-PDK1/FoxO1DKO mice among genes with an expression signal of >30 in control mice.

| Gene symbol | Gene accession | Probe set ID | Control signal | KO signal | $\log_2(\text{KO}/\text{control})$ | DKO signal | $\log_2(\text{DKO}/\text{control})$ |
|---------------|--------------------|--------------|----------------|-----------|------------------------------------|------------|-------------------------------------|
| Irs1 | NM_010570 | 17214753 | 33.6 | 21.3 | -0.66 | 43.5 | 0.37 |
| Gm5258 | XM_357111 | 17215101 | 34 | 24 | -0.5 | 40.4 | 0.25 |
| Mir3962 | NR_039539 | 17221010 | 40.5 | 27.4 | -0.57 | 44.5 | 0.13 |
| Abcb6 | ENSMUST00000027396 | 17224476 | 101.9 | 71.7 | -0.51 | 101 | -0.01 |
| BC035947 | BC138135 | 17224724 | 69.1 | 32.8 | -1.08 | 104.3 | 0.59 |
| Mndal | NM_001170853 | 17230067 | 61 | 36.4 | -0.74 | 53.4 | -0.19 |
| Mir1981 | NR_035502 | 17230750 | 65.2 | 39.2 | -0.73 | 57.5 | -0.18 |
| Epm2a | ENSMUST00000069106 | 17231669 | 52.9 | 32 | -0.73 | 49.7 | -0.09 |
| Vnn3 | NM_011979 | 17232152 | 102.3 | 69.5 | -0.56 | 97.4 | -0.07 |
| Echdc1 | NM_025855 | 17232426 | 429.7 | 294.1 | -0.55 | 404.4 | -0.09 |
| Rtn4ip1 | NM_130892 | 17233039 | 60.8 | 42 | -0.53 | 57.3 | -0.08 |
| Gnptab | NM_001004164 | 17236339 | 296.6 | 208.1 | -0.51 | 269.2 | -0.14 |
| G630090E17Rik | NM_001173500 | 17240303 | 34 | 22.9 | -0.57 | 34.1 | 0 |
| Prdm1 | NM_007548 | 17240645 | 37.5 | 26 | -0.53 | 49 | 0.39 |
| Slc16a7 | ENSMUST00000063318 | 17245648 | 150.5 | 98 | -0.62 | 237.8 | 0.66 |
| B430319H21Rik | AK021026 | 17250849 | 60.9 | 27.5 | -1.15 | 61 | 0 |
| Fgf11 | ENSMUST00000102585 | 17264960 | 52.5 | 31.3 | -0.75 | 46.7 | -0.17 |
| Slc2a4 | NM_009204 | 17265096 | 390.6 | 224.6 | -0.8 | 417.9 | 0.1 |
| Inca1 | NM_001252485 | 17265365 | 112 | 73.4 | -0.61 | 123.1 | 0.14 |
| Pctp | ENSMUST00000020864 | 17267685 | 63 | 42.6 | -0.57 | 56.6 | -0.15 |
| C630004H02Rik | NM_175454 | 17271861 | 60.5 | 40.8 | -0.57 | 53.4 | -0.18 |
| Hpcal1 | NM_016677 | 17274310 | 106.6 | 72.2 | -0.56 | 104.5 | -0.03 |
| Gm5784 | AK149188 | 17274369 | 69.6 | 45.5 | -0.61 | 74.2 | 0.09 |
| Ap4s1 | NM_021710 | 17275392 | 57 | 39.2 | -0.54 | 49.9 | -0.19 |
| Fam84a | NM_029007 | 17280041 | 36.1 | 23.3 | -0.63 | 34.5 | -0.07 |
| Ifi2712a | NM_029803 | 17283549 | 1669.5 | 952.3 | -0.81 | 2138.6 | 0.36 |
| Igh-VJ558 | BC031703 | 17284314 | 101.6 | 69 | -0.56 | 122.3 | 0.27 |
| Ighm | AB067787 | 17284360 | 1162.6 | 684.2 | -0.76 | 1463.2 | 0.33 |
| Ighv1-53 | AB069917 | 17284577 | 79.5 | 35.4 | -1.17 | 82.3 | 0.05 |
| Zfp493 | NM_028402 | 17288338 | 33.7 | 23 | -0.55 | 32.6 | -0.05 |
| Mblac2 | NM_028372 | 17288903 | 124.4 | 83.6 | -0.57 | 108.7 | -0.19 |
| Ankra2 | NM_023472 | 17289396 | 45 | 31.8 | -0.5 | 47.7 | 0.08 |
| Aldh5a1 | NM_172532 | 17291361 | 121.7 | 76 | -0.68 | 117.5 | -0.05 |
| Rpp40 | ENSMUST00000171686 | 17291854 | 50.5 | 35.2 | -0.52 | 50.6 | 0 |
| Muted | NM_139063 | 17291953 | 64.9 | 43.7 | -0.57 | 57.6 | -0.17 |
| Dtnbp1 | NM_025772 | 17292157 | 265 | 172 | -0.62 | 253.5 | -0.06 |
| Fgf10 | NM_008002 | 17296483 | 165.7 | 101.3 | -0.71 | 145.8 | -0.19 |
| Ero1l | NM_015774 | 17299196 | 56.6 | 35.3 | -0.68 | 51.1 | -0.15 |
| Trav9d-3 | X01134 | 17300247 | 77.9 | 48.7 | -0.68 | 120.2 | 0.63 |
| Fhit | ENSMUST00000160340 | 17303474 | 41.1 | 28.1 | -0.55 | 39.6 | -0.05 |
| Efs | NM_010112 | 17306524 | 41.8 | 25.4 | -0.72 | 46.2 | 0.14 |
| Prlr | NM_011169 | 17310259 | 60.9 | 34.1 | -0.84 | 74.9 | 0.3 |
| Cmb1 | NM_181588 | 17310816 | 599.8 | 374.3 | -0.68 | 571.2 | -0.07 |
| 4930572J05Rik | NM_198607 | 17312191 | 62.3 | 41.3 | -0.59 | 57.1 | -0.13 |
| Gcat | NM_013847 | 17312884 | 34.1 | 21 | -0.7 | 36 | 0.08 |
| 9330020H09Rik | NR_028442 | 17314626 | 32.6 | 19.6 | -0.74 | 28.9 | -0.17 |
| Hoxc4 | NM_013553 | 17315538 | 45.7 | 32.3 | -0.5 | 47.5 | 0.06 |
| Angpt1 | ENSMUST00000022921 | 17316780 | 216.6 | 124.9 | -0.79 | 227.6 | 0.07 |
| Oplah | ENSMUST00000171340 | 17318428 | 252.5 | 174.8 | -0.53 | 244.9 | -0.04 |
| Tst | NM_009437 | 17318983 | 313.3 | 185.8 | -0.75 | 313.9 | 0 |
| Prodh | ENSMUST00000003620 | 17328842 | 107.3 | 71.6 | -0.58 | 125.1 | 0.22 |
| Iglv1 | M94350 | 17329023 | 107.7 | 17 | -2.66 | 269.2 | 1.32 |
| Gm15760 | NR_030670 | 17329149 | 65.2 | 43.3 | -0.59 | 65.7 | 0.01 |
| Ehhadh | NM_023737 | 17329220 | 216 | 122.3 | -0.82 | 209.2 | -0.05 |
| Cyp4f17 | NM_001101445 | 17335952 | 213.5 | 121.5 | -0.81 | 235 | 0.14 |
| Cipp | NM_017393 | 17338925 | 171.9 | 116.7 | -0.56 | 172 | 0 |
| Mir219-1 | NR_029800 | 17343733 | 36.1 | 23.2 | -0.64 | 31.5 | -0.2 |
| LOC100862363 | XR_141834 | 17345004 | 33.6 | 22.9 | -0.55 | 39 | 0.21 |
| Lrg1 | NM_029796 | 17346150 | 738.9 | 474.5 | -0.64 | 681 | -0.12 |
| Mapre2 | NM_001162941 | 17348933 | 143.4 | 98.3 | -0.55 | 126.2 | -0.18 |
| Gm4013 | NR_033452 | 17354071 | 203 | 138.9 | -0.55 | 187.6 | -0.11 |
| Fibp | NM_001253832 | 17356434 | 156.4 | 110.5 | -0.5 | 137.6 | -0.18 |
| Cd274 | NM_021893 | 17358544 | 138.7 | 90.5 | -0.62 | 168.9 | 0.28 |
| O3far1 | NM_181748 | 17359113 | 101.3 | 69.7 | -0.54 | 97.2 | -0.06 |
| Gpam | ENSMUST00000086868 | 17365728 | 1674.5 | 1045.1 | -0.68 | 1663.3 | -0.01 |
| Ivd | ENSMUST00000028807 | 17374648 | 537 | 354.5 | -0.6 | 483.6 | -0.15 |
| Bcl2l11 | NM_207680 | 17375961 | 46.9 | 30.7 | -0.61 | 45.3 | -0.05 |
| Pygb | ENSMUST00000045441 | 17377498 | 846.2 | 591.5 | -0.52 | 830.3 | -0.03 |

Table S2-continued

| Gene symbol | Gene accession | Probe set ID | Control signal | KO signal | Log ₂ (KO/control) | DKO signal | Log ₂ (DKO/control) |
|---------------|--------------------|--------------|----------------|-----------|-------------------------------|------------|--------------------------------|
| Rbm38 | ENSMUST00000173393 | 17380199 | 103.1 | 71.6 | -0.53 | 94.7 | -0.12 |
| Gm14391 | ENSMUST00000151240 | 17380529 | 168.2 | 97.7 | -0.78 | 191.3 | 0.19 |
| Hnmt | NM_080462 | 17382203 | 329.5 | 222.5 | -0.57 | 327.4 | -0.01 |
| Olf1226 | NM_146967 | 17387912 | 36.1 | 23.2 | -0.64 | 41.3 | 0.19 |
| Tmem154 | NM_177260 | 17398571 | 72.8 | 50.8 | -0.52 | 69.6 | -0.07 |
| Gbp3 | ENSMUST00000029935 | 17403237 | 85.6 | 46.9 | -0.87 | 74.9 | -0.19 |
| Bche | NM_009738 | 17405908 | 334.2 | 210 | -0.67 | 298.7 | -0.16 |
| Dcst1 | NM_029974 | 17407049 | 53.5 | 37.6 | -0.51 | 48.8 | -0.13 |
| Rbm12b | NM_028226 | 17411892 | 42.8 | 18.9 | -1.18 | 41.7 | -0.04 |
| Tmem38b | NM_028053 | 17414250 | 54.4 | 37.1 | -0.55 | 50.1 | -0.12 |
| Gm12824 | NM_001085549 | 17417090 | 228.3 | 150.8 | -0.6 | 229.3 | 0.01 |
| Mycbp | ENSMUST00000030400 | 17418241 | 263.8 | 180.1 | -0.55 | 239.8 | -0.14 |
| Mecr | NM_025297 | 17419332 | 85.3 | 57.5 | -0.57 | 77.1 | -0.15 |
| Hnrph2 | NM_019868 | 17421170 | 49.2 | 34.7 | -0.5 | 43.9 | -0.17 |
| Gm13152 | ENSMUST00000063704 | 17421491 | 32.2 | 18 | -0.84 | 28.1 | -0.2 |
| 2810432D09Rik | NM_027278 | 17424293 | 53.2 | 35.7 | -0.57 | 52.8 | -0.01 |
| Gm568 | BC028561 | 17425120 | 49 | 31.9 | -0.62 | 43.8 | -0.16 |
| Bnc2 | NM_172870 | 17426929 | 339.5 | 211 | -0.69 | 327.7 | -0.05 |
| Gm12597 | NM_206870 | 17427067 | 33.6 | 23.8 | -0.5 | 33.3 | -0.01 |
| Cdkn2a | NM_009877 | 17427147 | 41.4 | 27.3 | -0.6 | 36.9 | -0.16 |
| Cdkn2c | NM_007671 | 17428217 | 266.3 | 143.9 | -0.89 | 284.6 | 0.1 |
| Zbtb48 | NM_133879 | 17433412 | 49.8 | 33.1 | -0.59 | 49.3 | -0.02 |
| Ras111b | NM_026878 | 17438189 | 68.7 | 38.8 | -0.82 | 61.3 | -0.16 |
| Igj | NM_152839 | 17449447 | 34.7 | 17.2 | -1.02 | 63.9 | 0.88 |
| Cdkl2 | ENSMUST00000086978 | 17449609 | 50.7 | 34.4 | -0.56 | 47.5 | -0.1 |
| Agpat9 | ENSMUST00000092990 | 17450196 | 149.9 | 83.7 | -0.84 | 167 | 0.16 |
| AB010352 | AB010352 | 17450950 | 41.8 | 28.6 | -0.55 | 46.1 | 0.14 |
| Try4 | NM_011646 | 17457731 | 63.8 | 41.6 | -0.62 | 58.2 | -0.13 |
| Plekha8 | NM_001164361 | 17458734 | 49.2 | 32 | -0.62 | 44.5 | -0.14 |
| Retsat | ENSMUST00000070597 | 17459676 | 416.5 | 255.2 | -0.71 | 399 | -0.06 |
| Asns | ENSMUST00000031766 | 17464718 | 317.5 | 214.4 | -0.57 | 331.2 | 0.06 |
| Igkw4-91 | X88903 | 17467398 | 69.8 | 36.9 | -0.92 | 78.7 | 0.17 |
| Elmod3 | NM_144917 | 17467782 | 322.2 | 197.4 | -0.71 | 291.9 | -0.14 |
| Wbp1 | ENSMUST00000032111 | 17468102 | 204.9 | 136.4 | -0.59 | 186 | -0.14 |
| Gys2 | NM_145572 | 17472497 | 81.6 | 51.4 | -0.67 | 74.8 | -0.13 |
| Bhlhe41 | ENSMUST00000032386 | 17472760 | 82 | 57.5 | -0.51 | 89 | 0.12 |
| Isoc2a | NM_001101598 | 17473402 | 106.9 | 65.6 | -0.7 | 95.1 | -0.17 |
| Zfp28 | NM_175247 | 17473603 | 48.7 | 32.3 | -0.59 | 42.6 | -0.19 |
| Ptgir | NM_008967 | 17474217 | 33.5 | 21.9 | -0.61 | 37.9 | 0.18 |
| Il28b | NM_177396 | 17475863 | 42 | 28.2 | -0.58 | 38.4 | -0.13 |
| Dbp | ENSMUST00000080885 | 17477979 | 733.9 | 483.8 | -0.6 | 801.4 | 0.13 |
| Sez6l2 | ENSMUST00000123117 | 17483192 | 48.4 | 26.7 | -0.86 | 45.2 | -0.1 |
| Hcst | NM_011827 | 17489046 | 36.4 | 22.7 | -0.68 | 37.8 | 0.06 |
| Cpeb1 | NM_001252525 | 17492754 | 106.7 | 75 | -0.51 | 94.9 | -0.17 |
| Hbb-b1 | NM_008220 | 17494221 | 200 | 123.7 | -0.69 | 227.9 | 0.19 |
| Coro1a | ENSMUST00000106364 | 17496376 | 63.7 | 39.9 | -0.68 | 61.5 | -0.05 |
| Fadd | ENSMUST00000033394 | 17498461 | 63 | 43.1 | -0.55 | 55.4 | -0.18 |
| Gm15315 | NM_001177528 | 17499796 | 81.6 | 50.4 | -0.69 | 77.1 | -0.08 |
| Car7 | NM_053070 | 17504454 | 55.1 | 38.5 | -0.52 | 49.4 | -0.16 |
| Cog4 | NM_133973 | 17505648 | 483.9 | 322.6 | -0.59 | 482.5 | 0 |
| Ednra | NM_010332 | 17510685 | 552.5 | 354.4 | -0.64 | 500.1 | -0.14 |
| Ces1f | NM_144930 | 17511731 | 502.2 | 216.7 | -1.21 | 533.2 | 0.09 |
| Ddx28 | NM_028038 | 17512630 | 35.8 | 25 | -0.52 | 34.8 | -0.04 |
| Ldhd | NM_027570 | 17513135 | 185 | 120.8 | -0.61 | 180.8 | -0.03 |
| Gm6484 | NM_001080940 | 17515335 | 122.1 | 84.6 | -0.53 | 123.1 | 0.01 |
| 2310047B19Rik | NM_025870 | 17515371 | 47.3 | 32.9 | -0.52 | 56.2 | 0.25 |
| Opcml | ENSMUST00000115243 | 17515653 | 33.4 | 22.8 | -0.55 | 33.2 | -0.01 |
| 9030425E11Rik | ENSMUST00000034522 | 17516352 | 416.9 | 286.6 | -0.54 | 452.2 | 0.12 |
| C920006O11Rik | NR_040401 | 17519644 | 43.3 | 26.1 | -0.73 | 42.5 | -0.03 |
| Cish | ENSMUST00000085102 | 17521300 | 49.5 | 24.9 | -0.99 | 47.4 | -0.06 |
| Muc16 | AK003577 | 17524311 | 42 | 23.6 | -0.83 | 39.3 | -0.1 |
| Anln | NM_028390 | 17524969 | 75.3 | 50.7 | -0.57 | 66.8 | -0.17 |
| Rbm15b | NM_175402 | 17530802 | 43.4 | 29.4 | -0.56 | 38.1 | -0.19 |
| Gm19971 | XR_106302 | 17533823 | 62.7 | 44 | -0.51 | 67.5 | 0.11 |
| Plxna3 | NM_008883 | 17535841 | 31.7 | 22.3 | -0.5 | 32.1 | 0.02 |
| C1galt1c1 | NM_021550 | 17541217 | 56.2 | 39.5 | -0.51 | 56.6 | 0.01 |
| 2310047B19Rik | NM_025870 | 17548156 | 84.4 | 55.2 | -0.61 | 81.3 | -0.06 |
| 4930412F12Rik | AK015120 | 17548675 | 42.8 | 27.6 | -0.64 | 42.4 | -0.02 |
| Gm6625 | XM_003085369 | 17548690 | 298.4 | 210.4 | -0.5 | 328.2 | 0.14 |

Table S3. List of lipid mediators detectable by LC-MS/MS–based metabololipidomics.

| Arachidonic acid-origin | | | |
|----------------------------------|----------------------------------|---------------------|------------------|
| Prostanoids | Leukotrienes | Lipoxins | Pathway markers |
| PGE ₂ | LTB ₄ | LXA ₄ | 5-HETE |
| PGD ₂ | Δ6-trans-LTB ₄ | LXB ₄ | 12-HETE |
| PGF _{2α} | 12-epi-Δ6-trans-LTB ₄ | 5,15-diHETE | 15-HETE |
| 8-iso-PGF _{2α} | 5,12-diHETE | AT-LXA ₄ | Arachidonic acid |
| 6-keto-PGI ₂ | 5,6-diHETE | AT-LXB ₄ | |
| PGJ ₂ | 20-OH-LTB ₄ | | |
| Δ12-PGJ ₂ | 20-COOH-LTB ₄ | | |
| 15-deoxy-Δ12,14-PGJ ₂ | LTC ₄ | | |
| TxB ₂ | LTD ₄ | | |
| | LTE ₄ | | |

| DHA-origin | | | |
|--------------------|----------------------|----------------------|-----------------|
| D-series resolvins | Protectin | Maresin | Pathway markers |
| RvD1 | PD1 | MaR1 | 4-HDHA |
| RvD2 | AT-PD1 | Δ12-trans-MaR1 | 7-HDHA |
| RvD3 | Δ15-trans-PD1 | 7-epi-Δ12-trans-MaR1 | 14-HDHA |
| RvD4 | 10-epi-Δ15-trans-PD1 | 4,14-diHDHA | 17-HDHA |
| RvD5 | 10,17-diHDHA | 7,14-diHDHA | DHA |
| RvD6 | | 13,14-diHDHA | |
| AT-RvD1 | | | |
| AT-RvD2 | | | |
| AT-RvD3 | | | |
| AT-RvD4 | | | |
| AT-RvD5 | | | |

| EPA-origin | | | | |
|-------------------|------------------|------------------|--------------------|-----------------|
| Prostanoids | Leukotrienes | Lipoxins | E-series resolvins | Pathway markers |
| PGE ₃ | LTB ₅ | LXA ₅ | RvE1 | 5-HEPE |
| PGD ₃ | LTC ₅ | LXB ₅ | RvE2 | 12-HEPE |
| PGF _{3α} | LTD ₅ | 5,15-diHEPE | RvE3 | 15-HEPE |
| TxB ₃ | LTE ₅ | | | 18-HEPE |
| | | | | EPA |