Supplementary Information

Oxidized Arachidonic/Adrenic Phosphatidylethanolamines Navigate Cells to Ferroptosis.

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Supplementary Table 1. List of potential death signals and correlation coefficients after application of the sifting criterion 2

PLox	Correlation coefficient
PI-(C18:0/C20:4+3[O])	0.850
PC-(C18:0/C20:4+3[O])	0.840
PE-(C18:1/C18:2+2[O])	0.806
PE-(C18:0/C22:4+2[O])	0.796
PS-(C18:0/C22:4+3[O])	0.780
PE-(C18:0/C22:4+1[O])	0.780
PE-(C18:0/C20:4+2[O])	0.772
PE-(C18:1/C20:4+3[O])	0.749
PE-(C18:1/C22:4+2[O])	0.745
PE-(C18:1/C20:4+2[O])	0.741
PC-(C18:0/C22:4+3[O])	0.736
PE-(C16:0/C20:4+3[O])	0.732
PE-(C18:1/C22:4+3[O])	0.731
PE-(C18:1/C22:4+1[O])	0.729
PE-(C18:0/C22:6+2[O])	0.716
PE-(C18:0/C20:4+3[O])	0.707
PE-(C18:0/C22:4+3[O])	0.702

Supplementary Table 2. For supporting supplementary figure 15, analysis of the phospholipid species interacting with 15LOX-2 at the final configuration (t = 1200 ns). The data show that PE species (SA-PE and alkenyl-SA-PE) are accountable for ~65% of total phospholipids interacting with the protein. None of SA-PC's present in the membrane interacts with the enzyme.

Lipids Present in the Bilayer	Total Number Of Lipids in Each Leaflet	Number of Lipids Interacting with LOX at 1200 ns	Percentage of the Interacting Lipids
PC (C16:0/C16:0)	135	5	29.4
pPC (C18:1/C18:2)	30	1	5.9
PC (C18:0/C20:4)	15	0	0
PE (C18:0/C20:4)	90	8	47.1
pPE (C18:0/C20:4)	30	3	17.6

Supplementary Table 3. Species description.

Species	Description	Initial Concentration (μM)
AA	Arachidonic acid	1
Acsl4	Long-chain-fatty-acid-CoA ligase 4	1
CoA	Coenzyme_A	100
5-HETE	5-Hydroxyicosatetraenoic acid	0.001
12-HETE	12-Hydroxyicosatetraenoic acid	0.001
15-HETE	15-Hydroxyicosatetraenoic acid	0.001
5-HpHETE	5-Hydroperoxyeicosatetraenoic acid	0.001
12-HpHETE	12-Hydroperoxyeicosatetraenoic acid	0.001
15-HpHETE	15-Hydroperoxyeicosatetraenoic acid	0.001
LOX5	lipoxygenase 5	5
LOX12	lipoxygenase 12	0.5
LOX15	lipoxygenase 15	1.5
AA-CoA	Arachidonoyl coenzyme A	1e-6
AdA	Adrenic acid	0.1
AdA-CoA	Adrenic acid coenzyme A	1e-6
COX	Cyclooxygenase	0.2
Cys	Cysteine	1
Cys-Glu	Glutamyl-cysteine	1
Elongase	Elongase	0.62
γGCS	Gamma-glutamylcysteine synthetase	1
Glu	Glutamtate	1
Gly	Glycine	1
Gpx4	Glutathione peroxidase 4	0.8
GR	Glutathione reductase	1
GS	Glutamine synthetase	1
GSH	Reduced glutathione	1
GSSH	Oxidized glutathione	1
Lcat3	Lecithin-cholesterol acyltransferase 3	1
LTA4	Leukotriene A4	0.001
LTA4H	Leukotriene A4 hydrolase,	0.76
LTB4	Leukotriene B4	0.001
lysoPE	Lysophosphatidylethanolamine	100
PE-AA	Phosphatidylethanolamine-adrenic acid	1e-6
PE-AA-OH	Phosphatidylethanolamin-arachidonoyl-hydroxy-derivative	1e-6
PE-AA-OOH	Phosphatidylethanolamin-arachidonoyl-hydroperoxy-derivative	1e-6
PE-AdA	Phosphatidylethanolamin-adrenic acid	1e-6
PE-AdA-OH	Phosphatidylethanolamin-adrenic acid- hydroxy-derivative	1e-6
PE-AdA-OOH	Phosphatidylethanolamin-adrenic acid- hydroperoxy-derivative	1e-6
PGE2	Prostaglandin E2	0.001
PGES	Prostaglandin E synthase	0.5
PGH2	Prostaglandin H2	0.001
PLA2	Phospholipase A2	0.0001
System-Xc	System xc- cystine/glutamate antiporter:	1
TXA2	Thromboxane A2	0.001
TXAS	Enzyme converts PGH2 to TXA2	0.5
w-LTB4	20-OH-Leukotriene B4	0.001
CYP4F3	Leukotriene B4 omega hydroxylase	0.07

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Supplementary Table 4. Reaction schema and differential equations for the phospholipid metabolic network regulating ferroptosis.

No.	Reaction
1	→ AA ; PLA2, 5-HETE, 12-HPETE, 15-HPETE, LTB4
2	AA → PGH2 ; COX, PGE2
3	AA → 5-HPETE ; LOX5, PGE2, 5-HETE, 12-HETE, 15-HETE
4	AA → 12-HPETE ; LOX12, 15-HETE
5	AA → 15-HPETE ; LOX15
6	5-HPETE + GSH → 5-HETE + GSSG; Gpx4
7	12-HPETE + GSH → 12-HETE + GSSG; Gpx4
8	15-HPETE + GSH → 15-HETE + GSSG; Gpx4
9	5-HPETE → LTA4; LOX5, PGE2, 5-HETE, 12-HETE, 15-HETE
10	LTA4 → LTB4; LTA4
11	LTB4 → w-LTB4; 5-HETE, 12-HETE
12	→ LOX5 ; LTA4
13	LTA4H → ; LTA4
14	LOX12 → ; 15-HPHETE
15	PGH → PGE2 ; PGES, AA, 15-HETE
16	AA → AA-CoA ; Ascl4
17	AA-CoA → AdA-CoA ; Elongase
18	AA-CoA → ; beta-oxidation-enzyme
19	AdA-CoA → ; beta-oxidation-enzyme
20	AA-CoA + lysoPE → PE-AA ; Lcat3
21	AdA-CoA + lysoPE → PE-AdA ; Lcat3
22	PE-AA → PE-AA-OOH ; LOX5
23	PE-AdA → PE-AdA-OOH ; LOX5
24	PE-AA → PE-AA-OOH ; LOX12
25	PE-AdA → PE-AdA-OOH ; LOX12
26	PE-AA → PE-AA-OOH ; LOX15
27	PE-AdA → PE-AdA-OOH ; LOX15
28	PE-AA-OOH → lysoPE ; PLA2
29	PE-AdA-OOH → lysoPE ; PLA2
30	PE-AA-OOH + GSH → PE-AA-OH + GSSH ; Gpx4
31	PE-AdA-OOH + GSH → PE-AdA-OH + GSSH ; Gpx4
32	GSSG → GSH ; GR
33	Cys-Glu + Gly → GSH ; GS
34	Cys + Glu → Cys-Glu ; γGCS
35	→ Cys ; System-xc
36	Glu → ; System-xc
37	AA-CoA → AA-OOH
38	AdA-CoA → AdA-OOH
39	AA-OOH + lysoPE → PE-AA-OOH
40	AdA-OOH + lysoPE → PE-AdA-OOH
	Assignment
	Cell death score = cell_death_basal + cell_death_scale * ([PE-AA-OOH]+[PE-AdA-OOH])

Supplementary Table 4 (cont.).

Differential Equations*

$$\frac{d([AA]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(PLA2)"\cdot[PLA2]\cdot lin\cdot\left(1+\frac{["12\text{-}HPETE"]}{KI19}+\frac{["15\text{-}HPETE"]}{KI20}+\frac{[LTB4]}{KI21}+\frac{["5\text{-}HETE"]}{KI22}\right)}{lin+"Km(PLA2)"\cdot\left(1+\frac{[AA]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0\cdot["15\text{-}LOX"]\cdot[AA]}{[AA]+"Km(15\text{-}LOX)"\cdot\left(1+\frac{["15\text{-}HPETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0\cdot["12\text{-}LOX"]\cdot[AA]}{[AA]+"Km(12\text{-}LOX)"\cdot\left(1+\frac{["12\text{-}HPETE"]}{ki18}+\frac{["15\text{-}HETE"]}{ki16}+\frac{["12\text{-}HPETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0.001\cdot["COX\text{-}2"]\cdot[AA]}{[AA]+"Km(COX\text{-}2)"\cdot\left(1+\frac{[PGE2]}{ki3}+\frac{[PGH2]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0\cdot["5\text{-}LOX"]\cdot[AA]}{[AA]+"Km(5\text{-}LOX)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ki7}+\frac{["15\text{-}HETE"]}{ki8}+\frac{[PGE2]}{ki11}+\frac{["5\text{-}HETE"]}{ki12}+\frac{["5\text{-}HPETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(0.0\cdot[AA])$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Ascl4)"\cdot[Ascl4]\cdot[AA]\cdot[CoA]}{"Km(AA)"\cdot"Km(CoA)"+[AA]\cdot"Km(AA)"+[CoA]\cdot"Km(CoA)"+[AA]\cdot[CoA]}\right)$$

$$\frac{d(["5\text{-}HPETE"]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+[GSH]+["5\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["5\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{0\cdot["5\text{-}LOX"]\cdot[AA]}{[AA]+"Km(5\text{-}LOX)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ki7}+\frac{["15\text{-}HETE"]}{ki8}+\frac{[PGE2]}{ki11}+\frac{["5\text{-}HETE"]}{ki12}+\frac{["5\text{-}HPETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0\cdot[GPX4]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+"Km(PHGPx)"\cdot\left(1+\frac{["5\text{-}HETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(5\text{-}LOX)"\cdot["5\text{-}LOX"]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+"Km(5\text{-}LOX)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ki7}+\frac{["15\text{-}HETE"]}{ki8}+\frac{[PGE2]}{ki11}+\frac{["5\text{-}HETE"]}{ki12}+\frac{[LTA4]}{ks}\right)}\right)$$

$$\frac{d(["5\text{-}HETE"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+[GSH]+["5\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["5\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{0\cdot[GPX4]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+"Km(PHGPx)"\cdot\left(1+\frac{["5\text{-}HETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(kd11\cdot["5\text{-}HETE"])$$

$$\frac{d([LTA4]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(5\text{-}LOX)"\cdot["5\text{-}LOX"]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+"Km(5\text{-}LOX)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ki7}+\frac{["15\text{-}HETE"]}{ki8}+\frac{[PGE2]}{ki11}+\frac{["5\text{-}HETE"]}{ki12}+\frac{[LTA4]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(LTA4H)"\cdot[LTA4H]\cdot[LTA4]}{[LTA4]+"Km(LTA4H)"\cdot\left(1+\frac{[LTB4]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(kd12\cdot[LTA4])$$

$$\frac{d([LTB4]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(LTA4H)"\cdot[LTA4H]\cdot[LTA4]}{[LTA4]+"Km(LTA4H)"\cdot\left(1+\frac{[LTB4]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(CYP4F3)"\cdot[CYP4F3]\cdot[LTB4]}{[LTB4]+"Km(CYP4F3)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ki14}+\frac{["5\text{-}HETE"]}{ki15}+\frac{["w\text{-}LTB4"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(kd13\cdot[LTB4])$$

$$\frac{d(["w\text{-}LTB4"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(CYP4F3)"\cdot[CYP4F3]\cdot[LTB4]}{[LTB4]+"Km(CYP4F3)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ki14}+\frac{["5\text{-}HETE"]}{ki15}+\frac{["w\text{-}LTB4"]}{ks}\right)}\right)$$

$$\frac{d(["15\text{-}LOX"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{0\cdot[PGE2]\cdot[PGE2]}{[PGE2]\cdot[PGE2]+KI24\cdot KI24}\right)$$
$$-V_{cell}\cdot(kd16\cdot["15\text{-}LOX"])$$

$$\frac{d(["12\text{-}LOX"]\cdot V_{cell})}{dt} = -V_{cell}\cdot(ki17\cdot["15\text{-}HPETE"]\cdot["12\text{-}LOX"])$$

Supplementary Table 4 (cont.).

Differential Equations

$$\frac{d(["15\text{-}HPETE"]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["15\text{-}HPETE"]}{["15\text{-}HPETE"]+[GSH]+["15\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["15\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{0\cdot["15\text{-}LOX"]\cdot[AA]}{[AA]+"Km(15\text{-}LOX)"\cdot\left(1+\frac{["15\text{-}HPETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0\cdot[GPX4]\cdot["15\text{-}HPETE"]}{["15\text{-}HPETE"]+"Km(PHGPx)"\cdot\left(1+\frac{["15\text{-}HETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(kd2\cdot["15\text{-}HPETE"])$$

$$\frac{d([TXAS]\cdot V_{cell})}{dt} = -V_{cell}\cdot(ki4\cdot["15\text{-}HPETE"]\cdot[TXAS])$$
$$-V_{cell}\cdot(ki5\cdot[PGH2]\cdot[TXAS])$$

$$\frac{d(["5\text{-}LOX"]\cdot V_{cell})}{dt} = +V_{cell}\cdot(KI23\cdot[LTB4]\cdot["5\text{-}LOX"])$$
$$-V_{cell}\cdot(ki9\cdot[LTA4]\cdot["5\text{-}LOX"])$$
$$-V_{cell}\cdot(ki10\cdot["5\text{-}HPETE"]\cdot["5\text{-}LOX"])$$
$$-V_{cell}\cdot(ki6\cdot["5\text{-}LOX"]\cdot["15\text{-}HPETE"])$$

$$\frac{d([LTA4H]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(LTA4H)"\cdot[LTA4H]\cdot[LTA4]}{([LTA4]+"Km(LTA4H)")\cdot 129}\right)$$

$$\frac{d(["15\text{-}HETE"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["15\text{-}HPETE"]}{["15\text{-}HPETE"]+[GSH]+["15\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["15\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{0\cdot[GPX4]\cdot["15\text{-}HPETE"]}{["15\text{-}HPETE"]+"Km(PHGPx)"\cdot\left(1+\frac{["15\text{-}HETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(kd3\cdot["15\text{-}HETE"])$$

$$\frac{d(["12\text{-}HPETE"]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["12\text{-}HPETE"]}{["12\text{-}HPETE"]+[GSH]+["12\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{0\cdot["12\text{-}LOX"]\cdot[AA]}{[AA]+"Km(12\text{-}LOX)"\cdot\left(1+\frac{["12\text{-}HPETE"]}{ki18}+\frac{["15\text{-}HETE"]}{ki16}+\frac{["12\text{-}HPETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{0\cdot[GPX4]\cdot["12\text{-}HPETE"]}{["12\text{-}HPETE"]+"Km(PHGPx)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ks}\right)}\right)$$

$$\frac{d(["12\text{-}HETE"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["12\text{-}HPETE"]}{["12\text{-}HPETE"]+[GSH]+["12\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{0\cdot[GPX4]\cdot["12\text{-}HPETE"]}{["12\text{-}HPETE"]+"Km(PHGPx)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ks}\right)}\right)$$

$$\frac{d([PGH2]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{0.001\cdot["COX\text{-}2"]\cdot[AA]}{[AA]+"Km(COX\text{-}2)"\cdot\left(1+\frac{[PGE2]}{ki3}+\frac{[PGH2]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(PGES)"\cdot[PGES]\cdot[PGH2]}{[PGH2]+"Km(PGES)"\cdot\left(1+\frac{[AA]}{ki1}+\frac{["15\text{-}HETE"]}{ki2}+\frac{[PGE2]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(TXAS)"\cdot[TXAS]\cdot[PGH2]}{[PGH2]+"Km(TXAS)"\cdot\left(1+\frac{[TXA2]}{ks}\right)}\right)$$

$$\frac{d([PGE2]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(PGES)"\cdot[PGES]\cdot[PGH2]}{[PGH2]+"Km(PGES)"\cdot\left(1+\frac{[AA]}{ki1}+\frac{["15\text{-}HETE"]}{ki2}+\frac{[PGE2]}{ks}\right)}\right)$$

$$\frac{d([TXA2]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(TXAS)"\cdot[TXAS]\cdot[PGH2]}{[PGH2]+"Km(TXAS)"\cdot\left(1+\frac{[TXA2]}{ks}\right)}\right)$$
$$-V_{cell}\cdot(kd8\cdot[TXA2])$$

$$\frac{d([CoA]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(Ascl4)"\cdot[Ascl4]\cdot[AdA]\cdot[CoA]}{"Km(AA)"\cdot"Km(CoA)"+[AdA]\cdot"Km(AA)"+[CoA]\cdot"Km(CoA)"+[AdA]\cdot[CoA]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Ascl4)"\cdot[Ascl4]\cdot[AA]\cdot[CoA]}{"Km(AA)"\cdot"Km(CoA)"+[AA]\cdot"Km(AA)"+[CoA]\cdot"Km(CoA)"+[AA]\cdot[CoA]}\right)$$

$$\frac{d(["AA\text{-}CoA"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(Ascl4)"\cdot[Ascl4]\cdot[AA]\cdot[CoA]}{"Km(AA)"\cdot"Km(CoA)"+[AA]\cdot"Km(AA)"+[CoA]\cdot"Km(CoA)"+[AA]\cdot[CoA]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Elongase)"\cdot[Elongase]\cdot["AA\text{-}CoA"]}{"Km(AA\text{-}CoA)\_e"+["AA\text{-}CoA"]}\right)$$
$$-V_{cell}\cdot("beta\text{-}oxidation"\cdot["AA\text{-}CoA"])$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Lcat3)"\cdot[Lcat3]\cdot["AA\text{-}CoA"]\cdot[lysoPE]}{"Km(AA\text{-}CoA)\_l"\cdot"Km(lysoPE)"+["AA\text{-}CoA"]\cdot"Km(AA\text{-}CoA)\_l"+[lysoPE]\cdot"Km(lysoPE)"+["AA\text{-}CoA"]\cdot[lysoPE]}\right)$$

$$\frac{d(["AdA\text{-}CoA"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(Ascl4)"\cdot[Ascl4]\cdot[AdA]\cdot[CoA]}{"Km(AA)"\cdot"Km(CoA)"+[AdA]\cdot"Km(AA)"+[CoA]\cdot"Km(CoA)"+[AdA]\cdot[CoA]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(Elongase)"\cdot[Elongase]\cdot["AA\text{-}CoA"]}{"Km(AA\text{-}CoA)\_e"+["AA\text{-}CoA"]}\right)$$
$$-V_{cell}\cdot("beta\text{-}oxidation"\cdot["AdA\text{-}CoA"])$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Lcat3)"\cdot[Lcat3]\cdot["AdA\text{-}CoA"]\cdot[lysoPE]}{"Km(AA\text{-}CoA)\_l"\cdot"Km(lysoPE)"+["AdA\text{-}CoA"]\cdot"Km(AA\text{-}CoA)\_l"+[lysoPE]\cdot"Km(lysoPE)"+["AdA\text{-}CoA"]\cdot[lysoPE]}\right)$$

$$\frac{d(["PE\text{-}AA"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(Lcat3)"\cdot[Lcat3]\cdot["AA\text{-}CoA"]\cdot[lysoPE]}{"Km(AA\text{-}CoA)\_l"\cdot"Km(lysoPE)"+["AA\text{-}CoA"]\cdot"Km(AA\text{-}CoA)\_l"+[lysoPE]\cdot"Km(lysoPE)"+["AA\text{-}CoA"]\cdot[lysoPE]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(15\text{-}LOX)\_o"\cdot["15\text{-}LOX"]\cdot["PE\text{-}AA"]}{"Km(PE)"+["PE\text{-}AA"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(12\text{-}LOX)\_o"\cdot["12\text{-}LOX"]\cdot["PE\text{-}AA"]}{"Km(PE)"+["PE\text{-}AA"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(5\text{-}LOX)\_o"\cdot["5\text{-}LOX"]\cdot["PE\text{-}AA"]}{"Km(PE)"+["PE\text{-}AA"]}\right)$$

Supplementary Table 4 (cont.).

Differential Equations

$$\frac{d([lysoPE]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(PLA2)\_l"\cdot[PLA2]\cdot["PE\text{-}AA\text{-}OOH"]}{"Km(PE\text{-}OOH)\_l"+["PE\text{-}AA\text{-}OOH"]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(PLA2)\_l"\cdot[PLA2]\cdot["PE\text{-}AdA\text{-}OOH"]}{"Km(PE\text{-}OOH)\_l"+["PE\text{-}AdA\text{-}OOH"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Lcat3)"\cdot[Lcat3]\cdot["AA\text{-}CoA"]\cdot[lysoPE]}{"Km(AA\text{-}CoA)\_l"\cdot"Km(lysoPE)"+["AA\text{-}CoA"]\cdot"Km(AA\text{-}CoA)\_l"+[lysoPE]\cdot"Km(lysoPE)"+["AA\text{-}CoA"]\cdot[lysoPE]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(Lcat3)"\cdot[Lcat3]\cdot["AdA\text{-}CoA"]\cdot[lysoPE]}{"Km(AA\text{-}CoA)\_l"\cdot"Km(lysoPE)"+["AdA\text{-}CoA"]\cdot"Km(AA\text{-}CoA)\_l"+[lysoPE]\cdot"Km(lysoPE)"+["AdA\text{-}CoA"]\cdot[lysoPE]}\right)$$

$$\frac{d(["PE\text{-}AdA"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(Lcat3)"\cdot[Lcat3]\cdot["AdA\text{-}CoA"]\cdot[lysoPE]}{"Km(AA\text{-}CoA)\_l"\cdot"Km(lysoPE)"+["AdA\text{-}CoA"]\cdot"Km(AA\text{-}CoA)\_l"+[lysoPE]\cdot"Km(lysoPE)"+["AdA\text{-}CoA"]\cdot[lysoPE]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(15\text{-}LOX)\_o"\cdot["15\text{-}LOX"]\cdot["PE\text{-}AdA"]}{"Km(PE)"+["PE\text{-}AdA"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(12\text{-}LOX)\_o"\cdot["12\text{-}LOX"]\cdot["PE\text{-}AdA"]}{"Km(PE)"+["PE\text{-}AdA"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(5\text{-}LOX)\_o"\cdot["5\text{-}LOX"]\cdot["PE\text{-}AdA"]}{"Km(PE)"+["PE\text{-}AdA"]}\right)$$

$$\frac{d(["PE\text{-}AA\text{-}OOH"]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(PLA2)\_l"\cdot[PLA2]\cdot["PE\text{-}AA\text{-}OOH"]}{"Km(PE\text{-}OOH)\_l"+["PE\text{-}AA\text{-}OOH"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(15\text{-}LOX)\_o"\cdot["15\text{-}LOX"]\cdot["PE\text{-}AA"]}{"Km(PE)"+["PE\text{-}AA"]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(12\text{-}LOX)\_o"\cdot["12\text{-}LOX"]\cdot["PE\text{-}AA"]}{"Km(PE)"+["PE\text{-}AA"]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(5\text{-}LOX)\_o"\cdot["5\text{-}LOX"]\cdot["PE\text{-}AA"]}{"Km(PE)"+["PE\text{-}AA"]}\right)$$

$$\frac{d(["PE\text{-}AdA\text{-}OOH"]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(PLA2)\_l"\cdot[PLA2]\cdot["PE\text{-}AdA\text{-}OOH"]}{"Km(PE\text{-}OOH)\_l"+["PE\text{-}AdA\text{-}OOH"]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(15\text{-}LOX)\_o"\cdot["15\text{-}LOX"]\cdot["PE\text{-}AdA"]}{"Km(PE)"+["PE\text{-}AdA"]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(12\text{-}LOX)\_o"\cdot["12\text{-}LOX"]\cdot["PE\text{-}AdA"]}{"Km(PE)"+["PE\text{-}AdA"]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(5\text{-}LOX)\_o"\cdot["5\text{-}LOX"]\cdot["PE\text{-}AdA"]}{"Km(PE)"+["PE\text{-}AdA"]}\right)$$

$$\frac{d([GSH]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(GR)"\cdot[GR]\cdot[GSSG]}{"Km(GSSG)"+[GSSG]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(GS)"\cdot[GS]\cdot["Cys\text{-}Glu"]\cdot[Gly]}{"Km(Cys\text{-}Glu)"\cdot"Km(Gly)"+["Cys\text{-}Glu"]\cdot"Km(Cys\text{-}Glu)"+[Gly]\cdot"Km(Gly)"+["Cys\text{-}Glu"]\cdot[Gly]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["12\text{-}HPETE"]}{["12\text{-}HPETE"]+[GSH]+["12\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["15\text{-}HPETE"]}{["15\text{-}HPETE"]+[GSH]+["15\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["15\text{-}HETE"]}{ks}\right)}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+[GSH]+["5\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["5\text{-}HETE"]}{ks}\right)}\right)$$

$$\frac{d([GSSG]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}\right)$$
$$-V_{cell}\cdot\left(\frac{"Kcat(GR)"\cdot[GR]\cdot[GSSG]}{"Km(GSSG)"+[GSSG]}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["12\text{-}HPETE"]}{["12\text{-}HPETE"]+[GSH]+["12\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["12\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["15\text{-}HPETE"]}{["15\text{-}HPETE"]+[GSH]+["15\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["15\text{-}HETE"]}{ks}\right)}\right)$$
$$+V_{cell}\cdot\left(\frac{"Kcat(PHGPx)"\cdot[GSH]\cdot[GPX4]\cdot["5\text{-}HPETE"]}{["5\text{-}HPETE"]+[GSH]+["5\text{-}HPETE"]\cdot[GSH]+"Km(PHGPx)"\cdot\left(1+\frac{["5\text{-}HETE"]}{ks}\right)}\right)$$

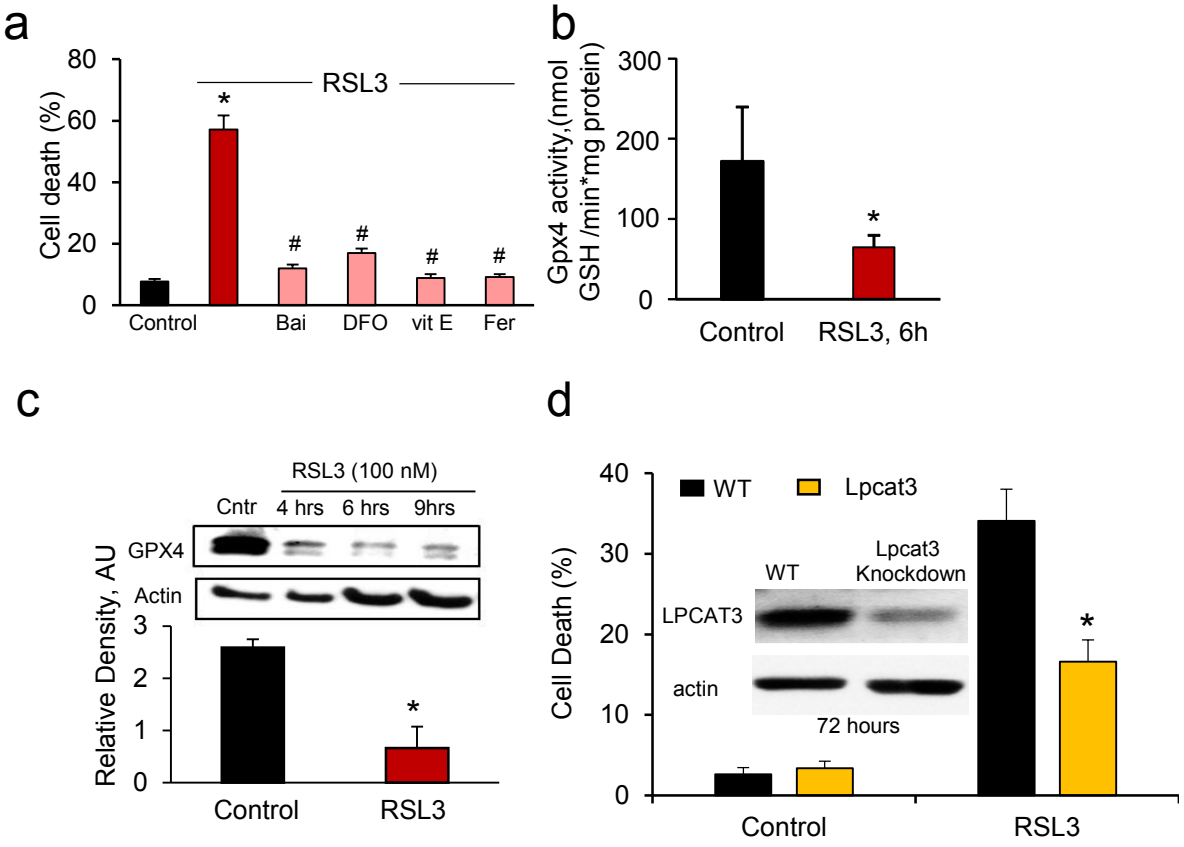
Supplementary Table 4 (cont.).

Differential Equations
$\frac{d(["PE\text{-}AA\text{-}OH"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AA\text{-}OOH"]\cdot[GSH]}\right) - V_{cell}\cdot(0.4\cdot["PE\text{-}AA\text{-}OH"])$
$\frac{d(["PE\text{-}AdA\text{-}OH"]\cdot V_{cell})}{dt} = +V_{cell}\cdot\left(\frac{"Kcat(GPX4)"\cdot[GPX4]\cdot["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}{"Km(PE\text{-}OOH)"\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot"Km(PE\text{-}OOH)"+[GSH]\cdot"Km(GSH)"+["PE\text{-}AdA\text{-}OOH"]\cdot[GSH]}\right) - V_{cell}\cdot(0.4\cdot["PE\text{-}AdA\text{-}OH"])$
$\frac{d(["Cys\text{-}Glu"]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(GS)"\cdot[GS]\cdot["Cys\text{-}Glu"]\cdot[Gly]}{"Km(Cys\text{-}Glu)"\cdot"Km(Gly)"+["Cys\text{-}Glu"]\cdot"Km(Cys\text{-}Glu)"+[Gly]\cdot"Km(Gly)"+["Cys\text{-}Glu"]\cdot[Gly]}\right) + V_{cell}\cdot\left(\frac{"Kcat(GCS)"\cdot[gammaGCS]\cdot[Cys]\cdot[Glu]}{"Km(Cys)"\cdot"Km(Glu)"+[Cys]\cdot"Km(Cys)"+[Glu]\cdot"Km(Glu)"+[Cys]\cdot[Glu]}\right)$
$\frac{d([Gly]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(GS)"\cdot[GS]\cdot["Cys\text{-}Glu"]\cdot[Gly]}{"Km(Cys\text{-}Glu)"\cdot"Km(Gly)"+["Cys\text{-}Glu"]\cdot"Km(Cys\text{-}Glu)"+[Gly]\cdot"Km(Gly)"+["Cys\text{-}Glu"]\cdot[Gly]}\right)$
$\frac{d([Cys]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(GCS)"\cdot[gammaGCS]\cdot[Cys]\cdot[Glu]}{"Km(Cys)"\cdot"Km(Glu)"+[Cys]\cdot"Km(Cys)"+[Glu]\cdot"Km(Glu)"+[Cys]\cdot[Glu]}\right) + V_{cell}\cdot(kCys)$
$\frac{d([Glu]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(GCS)"\cdot[gammaGCS]\cdot[Cys]\cdot[Glu]}{"Km(Cys)"\cdot"Km(Glu)"+[Cys]\cdot"Km(Cys)"+[Glu]\cdot"Km(Glu)"+[Cys]\cdot[Glu]}\right) - V_{cell}\cdot(kGlu\cdot[Glu])$
$\frac{d([AdA]\cdot V_{cell})}{dt} = -V_{cell}\cdot\left(\frac{"Kcat(Ascl4)"\cdot[Ascl4]\cdot[AdA]\cdot[CoA]}{"Km(AA)"\cdot"Km(CoA)"+[AdA]\cdot"Km(AA)"+[CoA]\cdot"Km(CoA)"+[AdA]\cdot[CoA]}\right)$

* The differential equations of constant species (i.e. d[x]/dt=0) is omitted in the table.

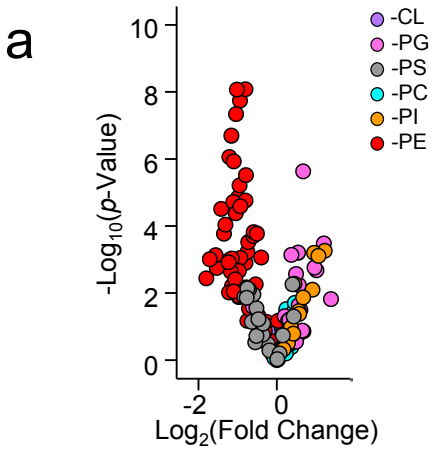
Supplementary Table 5. Model parameters.

Name	Value	Name	Value	Name	Value
lin	12	ki2	30	kdAA-CoA	0.01
Kcat(PLA2)	3.6	ki3	30	kdAdA-CoA	0.01
Km(PLA2)	2.6	ki4	0.6	Km(AA-CoA)_l	10
Kcat(15-LOX)	1000	ki5	0.1	Km(lysoPE)	10
Km(15-LOX)	70	ki6	0.01	Kcat(Lcat3)	1000
Kcat(12-LOX)	1000	ki7	30	kGlu	1
Km(12-LOX)	50	ki8	4	kCys	1
Kcat(COX-2)	1000	ki9	0.175	Kcat(GCS)	1000
Km(COX-2)	50	ki10	0.01	Km(Cys)	10
Kcat(PGES)	3000	ki11	15	Km(Glu)	10
Km(PGES)	160	ki12	6.3	Kcat(GS)	1000
Kcat(TXAS)	1599	ki14	0.2	Km(Cys-Glu)	10
Km(TXAS)	4	ki15	0.86	Km(Gly)	10
Kcat(5-LOX)	5000	ki16	10	Kcat(GR)	1000
Km(5-LOX)	5	ki17	10	Km(GSSG)	10
Kcat(LTA4H)	125	ki18	10	Kcat(GPX4)	1000
Km(LTA4H)	20	KI19	500	Km(PE-OOH)	10
Kcat(CYP4F3)	150	KI20	200	Km(GSH)	10
Km(CYP4F3)	3.9	KI21	500	Kcat(PLA2)_l	0.01
Kcat(PHGPx)	500	KI22	500	Km(PE-OOH)_l	10
Km(PHGPx)	70	KI23	0.053	Kcat(5-LOX)_o	100
kd2	0.05	KI24	2.30E-05	Kcat(12-LOX)_o	100
kd3	0.01	a24	0.15	Km(PE)	10
kd8	0.1	ks	500	Kcat(COX)	1000
kd9	0.001	Km(AA)	10	Kcat(15-LOX)_o	1000
kd11	0.001	Km(CoA)	10	Km(PE)_c	10
kd12	0.07	Kcat(Ascl4)	0.1	cell_death_basal	3
kd13	0.01	Km(AA-CoA)	10	cell_death_scale	45000
kd16	0.01	Km(AA-CoA)_e	10	beta-oxidation	100
ki1	0.3	Kcat(Elongase)	1000	Vcell	1



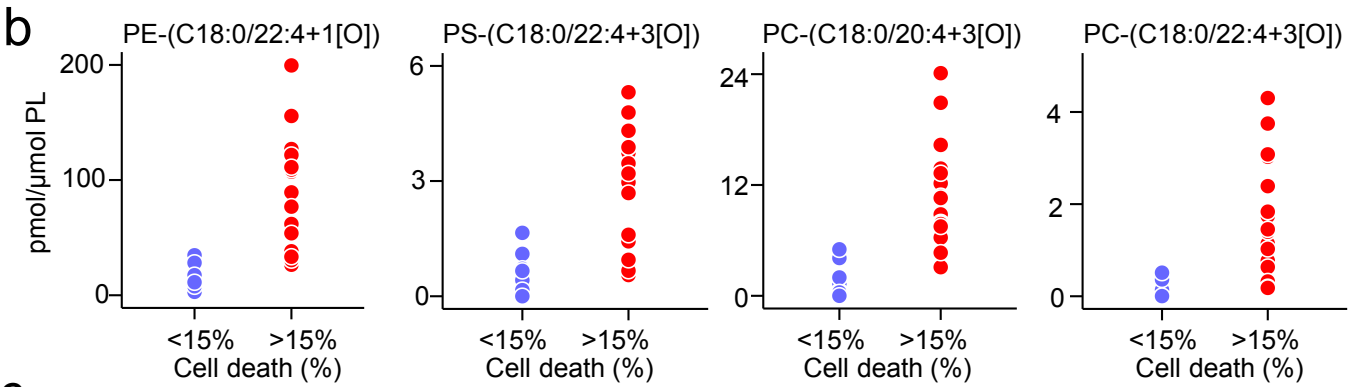
Supplementary Figure 1. RSL3-induced ferroptosis model and effect of Lpcat3 knockdown on ferroptosis in Pfa1 cells.

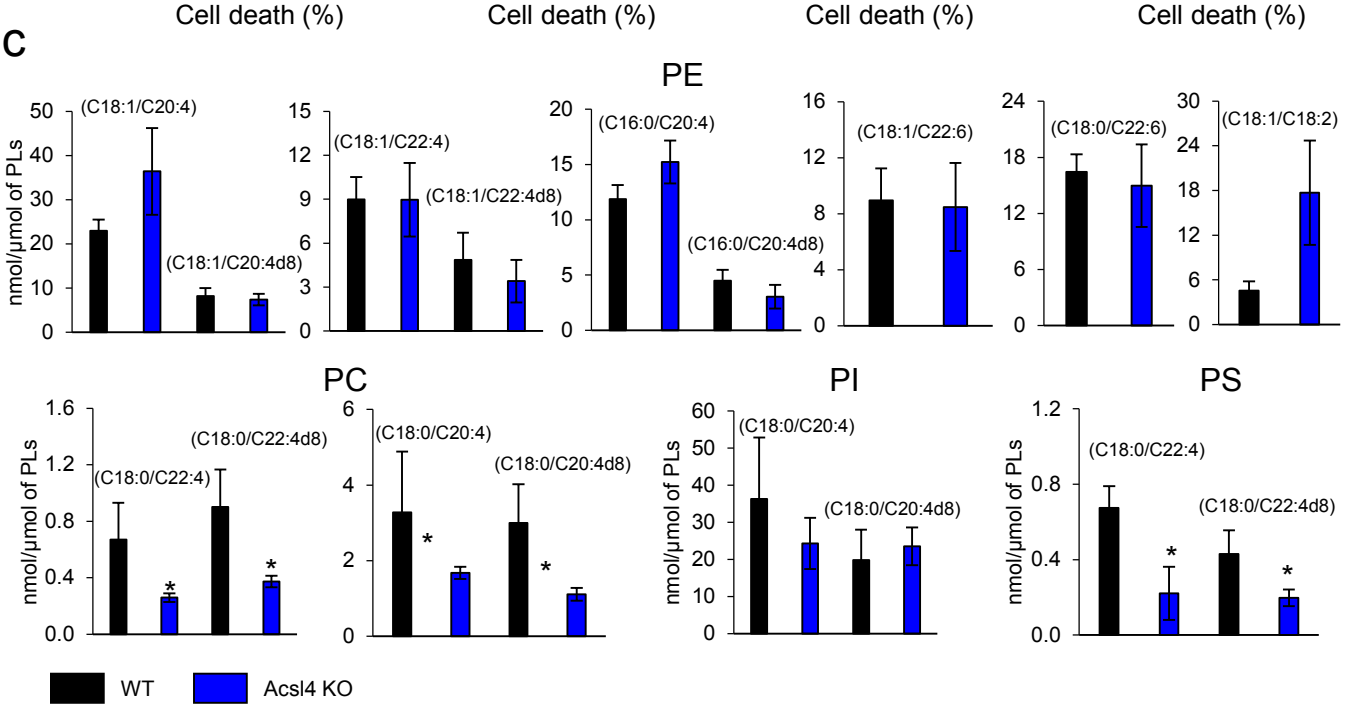
(**a**) Effects of inhibitors on RSL3 induced ferroptosis: 12/15-LOX inhibitor (Bai, Baicalein, 1μM), iron chelator (DFO, Deferoxamine, 10 μM), Vit E (α-tocopherol, 1μM), ferroptosis inhibitor (Fer, Ferrostatin-1, 0.1μM). Cells were incubated with 50 nM RSL3 for 18 hrs. Data are mean ± s.d., n=3. *, # indicates $P < 0.05$ v.s. control and RSL3, respectively. (**b**) GPX4 activity of naïve and RSL3-treated (100 nM, 6 hrs) Pfa1. Data are mean ± s.d., n=3. *$P < 0.05$ by t-test v.s. control. (**c**) Western blots of GPX4 after RSL3 treatment (100 nM) for 4, 6, 9 hrs (upper panel) and averaged quantitative measurements (6 hrs) of GPX4 content in Pfa1 cells (lower panel). Data are mean ± s.d., n=3. *$P < 0.05$ by t-test v.s. control. (**d**) Lpcat3 knock down decreases RSL3-induced ferroptosis in mouse embryonic fibroblasts. Data are mean ± s.d., n=3. *$P < 0.05$ by t-test v.s. WT+RSL3.

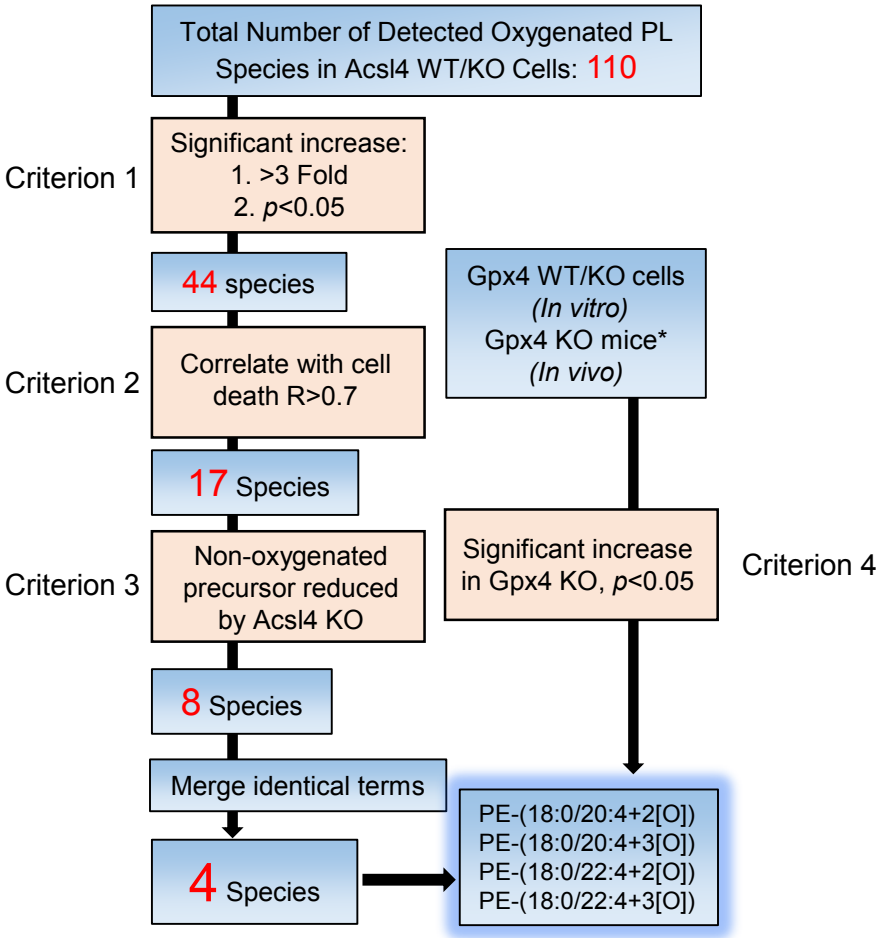


Supplementary Figure 2. Sifting ferroptotic lipidic death signals in Acsl4 WT/KO Pfa1 cells.

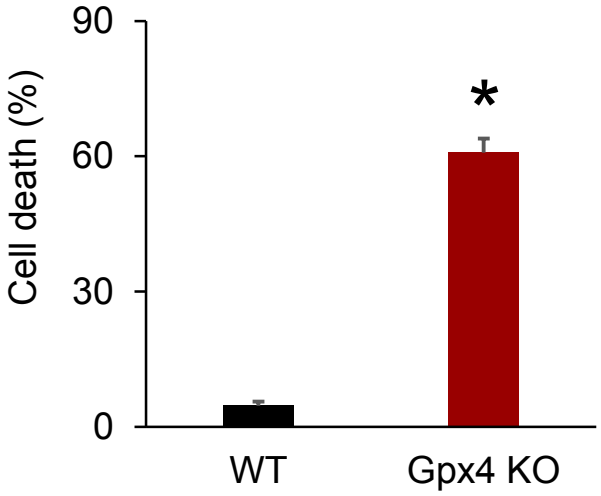
(a) Scatter plot of $\log_2$ fold-change on X-axis versus significance on Y-axis (by t-test) of live cells (death <15%, number of replicate data points is 26) and ferroptotic cells (death >15%, number of replicate data points is 18) for non-oxygenated phospholipids (PLs). **(b)** Contents of four oxygenated species of PLs (PE, PS, PC) as potential candidates for ferroptotic signals in live cells (death <15%) versus ferroptotic cells (death >15%). These species were sieved out after the application of criterion 3. **(c)** Levels of deuterated and non-deuterated non-oxygenated phospholipid precursors of seventeen oxygenated phospholipids sieved out after the application of criterion 3. Data are mean ± s. d., n=3. *P < 0.05 by t-test for Acsl4 KO cells (closed bars) v.s. WT cells (open bars).



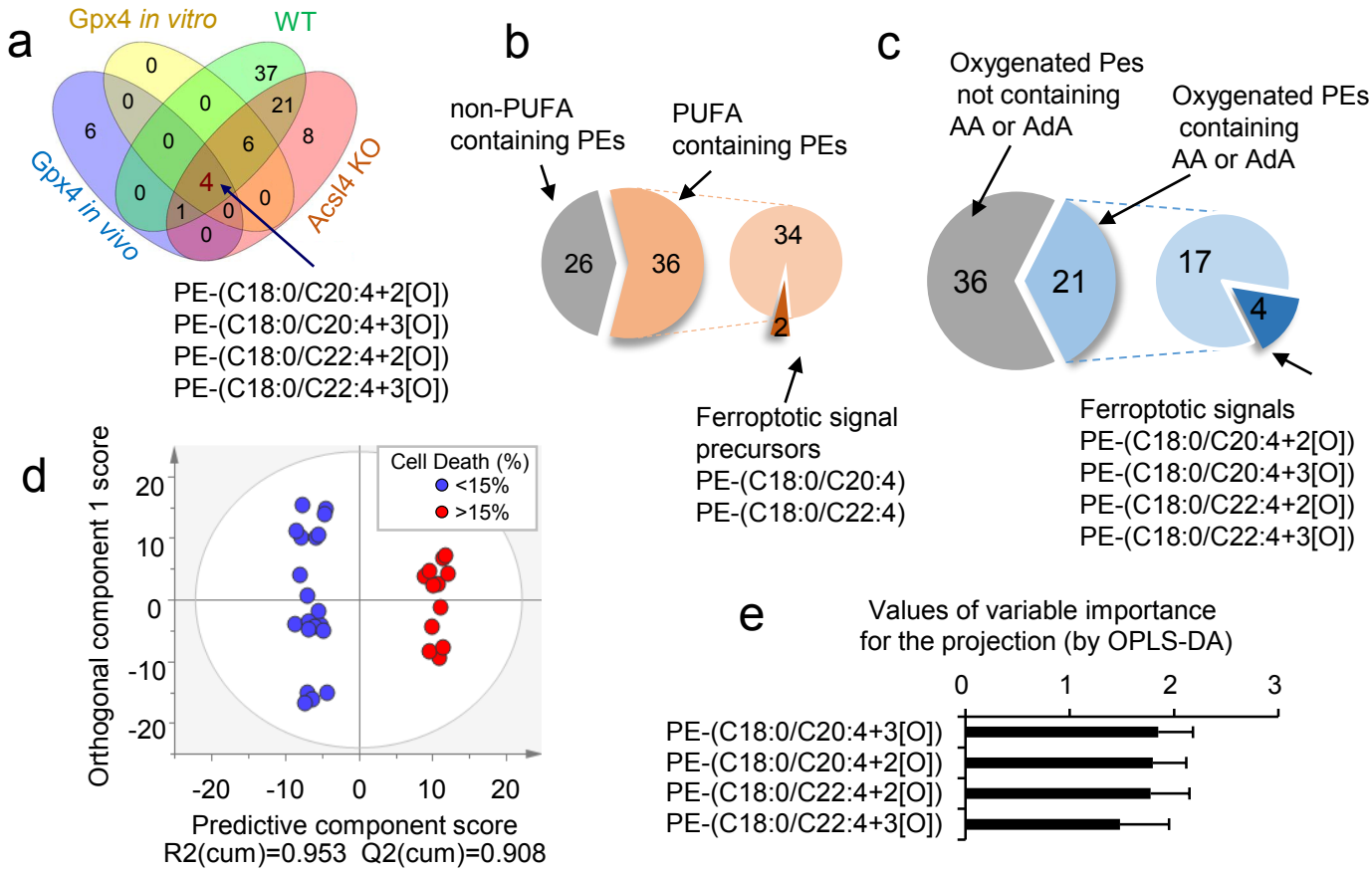




Supplementary Figure 3. Logical tree for screening and identifying phospholipids oxidation products as ferroptosis death signals in Acsl4 WT/KO and GPX4 WT/KO Pfa1 cells.

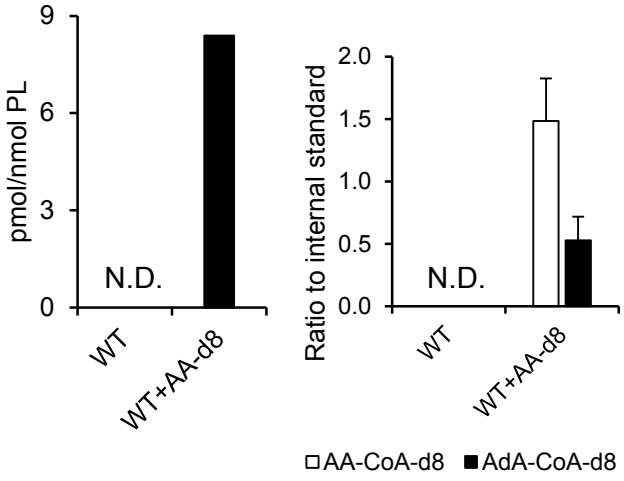


Supplementary Figure 4. Loss of GPX4 in Pfa1 cells caused cell death.
Cell death was assessed by PI staining at 50 hours following tamoxifen administration. Data are mean ± s. d. , n=3. *P < 0.05 by t-test for Gpx4 KO v.s. WT cells.



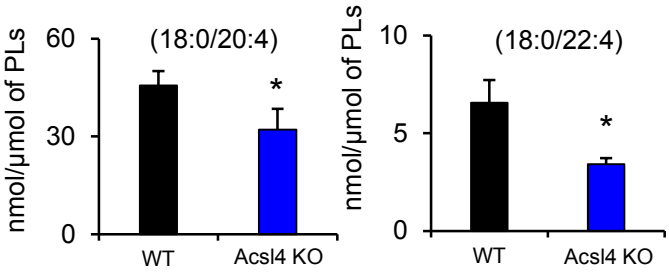
Supplementary Figure 5. Oxygenated PE species are identified in ferroptotic GPX4 deficient cells and kidney of GPX4-deficient mice.

(a) The four-way Venn diagram congregated oxygenated phospholipid species detected in Gpx4 KO Pfa1 cells *in vitro* and kidneys of Gpx4 KO mice as well as in RSL3 induced GPX4 deficient WT and Acsl4 KO Pfa1 cells treated with RSL3 into a four-member set of di- and tri-oxygenated PE-AA and PE-AdA species common to the function of ferroptotic signaling. The numbers in each area indicate the amounts of oxygenated phospholipids with significantly higher levels in ferroptotic group vs the respective control group by t-test. **(b)** High selectivity of PE oxygenation during ferroptosis: Total number of non-oxidizable (non-PUFA) plus oxidizable PUFA-PE species as precursors of PE-(C18:2/C20:4) and PE-(C18:0/C22:4) ferroptotic signals. **(c)** Total number of oxygenated PE species vs oxygenated AA- or AdA-containing PE species vs di- and tri-oxygenated PE species identified as ferroptotic signals. **(d)** Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot of the first two principal components of phospholipidome between levels of cell death < 15%, (number of replicate data points is N=26) and cell death >15% (number of replicate data points is N=18). Phospholipidomic data were mean-centered and UV-scaled. **(e)** Values of variable importance for the projection (by OPLS-DA) of potential biomarkers are all greater than 1. Standard error was derived from cross validation.



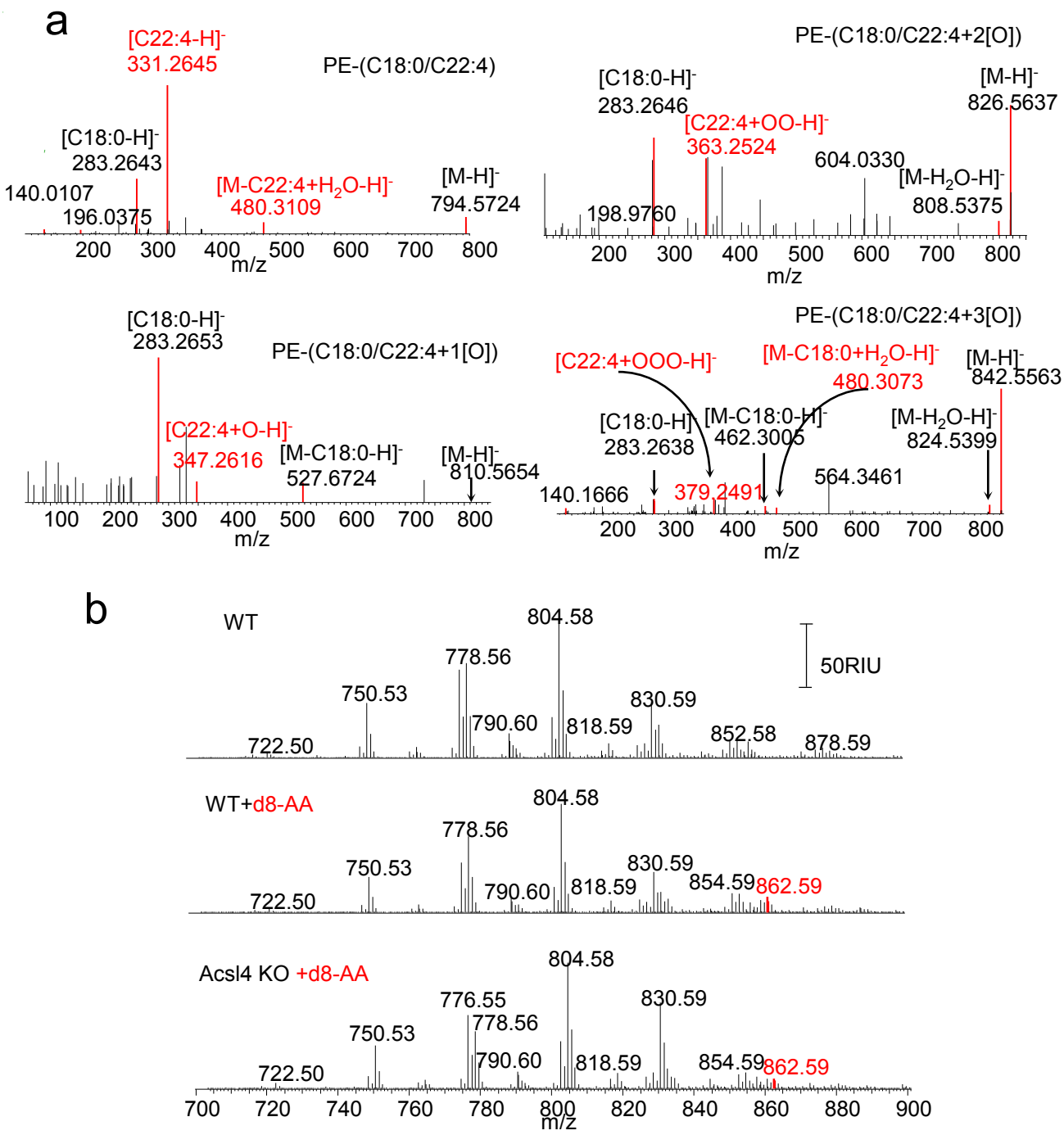
Supplementary Figure 6. Accumulation of free AdA-d8 (left panel), AA-CoA-d8 and AdA-CoA-d8 (right panel) after supplementation of Pfa1 cells with free AA-d8.

Data are mean ± s. d., n=3 (right panel) .



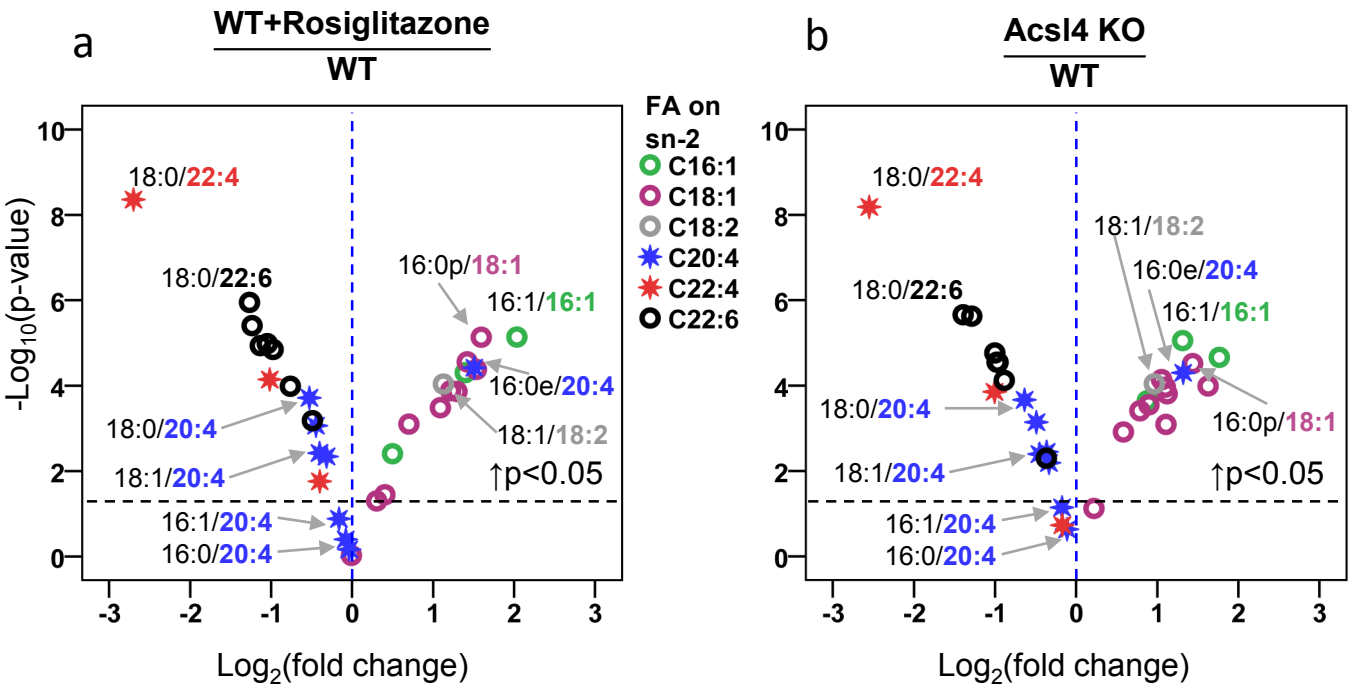
Supplementary Figure 7. Lower levels of PE-(C18:0/C20:4) (right panel) and PE-(C18:0/C22:4) (left panel) in Acsl4 KO compared to WT Pfa1 cells.

Data are mean ± s. d., n=3. *P < 0.05 by t-test v.s. WT cells.



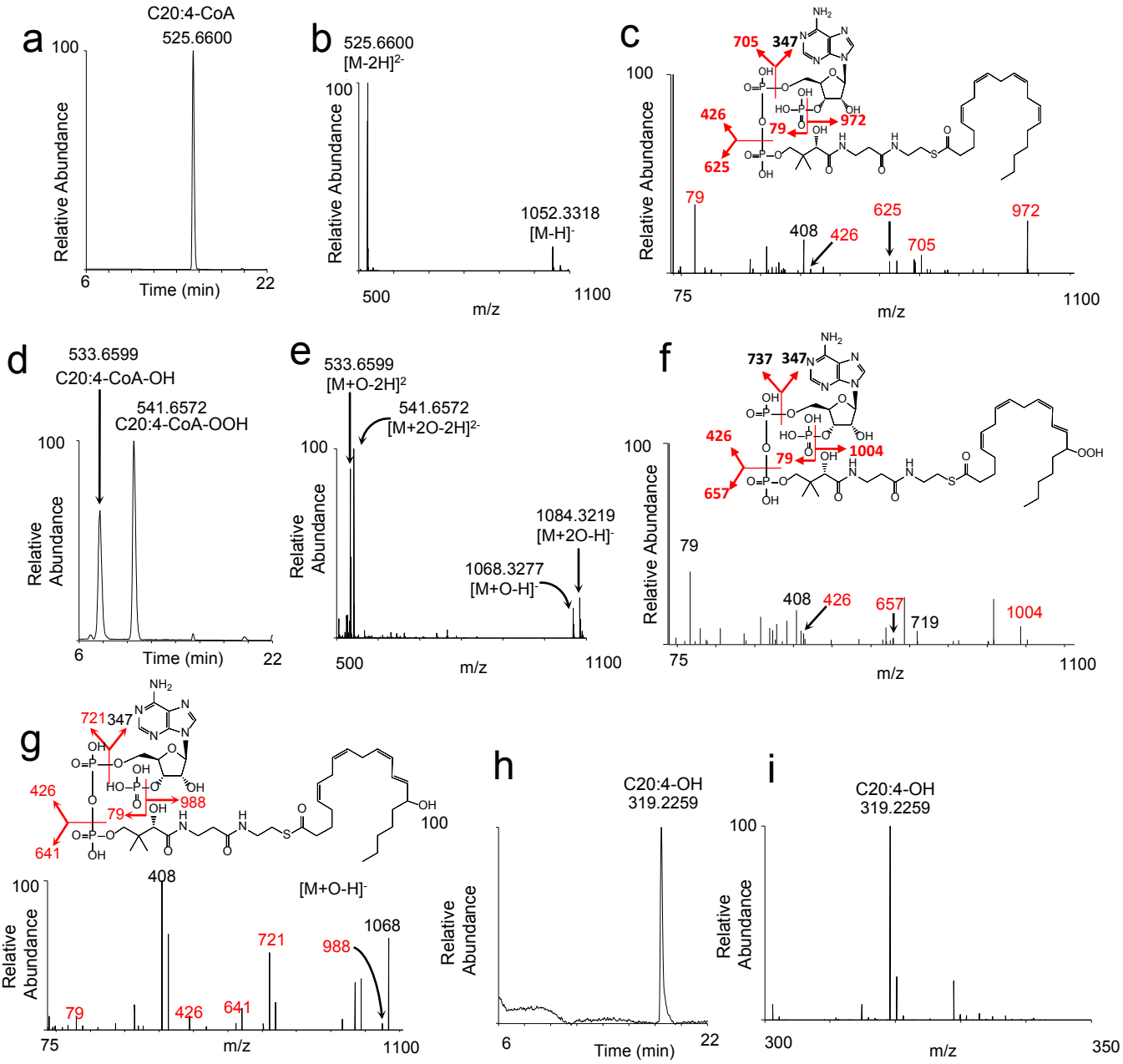
Supplementary Figure 8. Identification of ferroptotic lipidic death signals by LC-MS in Pfa1 cells.

(**a**) MS/MS spectra of PE-(C18:0/C22:4) and its oxygenated species with one, two and three oxygens detected in Pfa1 cells treated with RSL3; (**b**) Typical mass spectra of phosphatidylcholines (PC) from WT and Acsl4 KO cells loaded with deuterated AA. Note that only very small accumulation of *d8*-AA was detected in PC species (red) which was not affected by Acsl4 KO.



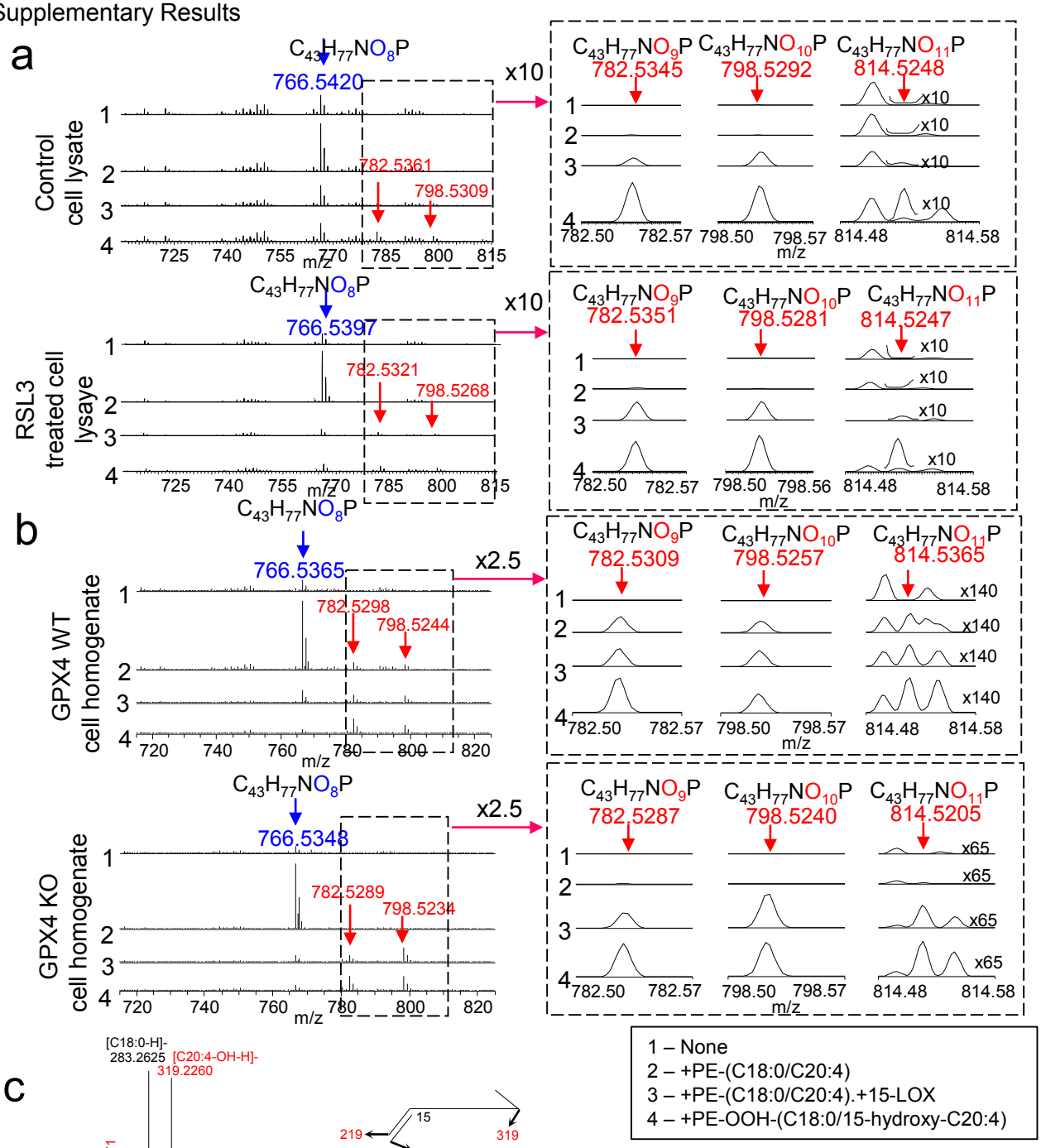
Supplementary Figure 9. Effect of Acsl4 KO and rosiglitazone on the content of oxidizable PE species in Pfa1 cells.

(a) Scatter plot of rosiglitazone induced changes in the levels of PE (log_2 (fold-change), X-axis) vs significance ($-log_{10}$(p-Value), Y-axis, by t-test) of WT and WT treated with Rosiglitazone Pfa1 cells (number of replicate data points is 3). **(b)** Scatter plot of Acsl4 KO induced changes in the levels of PE (log_2 (fold-change), X-axis) vs significance ($-log_{10}$(p-Value), Y-axis, by t-test) in Pfa1 cells (number of replicate data points is 3). Similar changes of PE profiles were observed in rosiglitazone treated cells and Acsl4 KO cells. These changes included decreased levels of ferroptotic signal precursors: PE-(C18:0/C20:4) and PE-(C18:0/C22:4).



Supplementary Figure 10. LC-MS analysis of C20:4-CoA and its oxidation products in a model system containing human recombinant 15-lipoxygenase (15-LOX-2), H_2O_2, $CaCl_2$ and DTPA in 25mM Hepes buffer (pH 7.4).

(**a**) Typical LC-MS chromatogram of non-oxidized C20:4-CoA. (**b**) Spectrum of non-oxidized C20:4-CoA displaying singly and doubly negative charged ions. (**c**) Fragmentation spectrum and structure of C20:4-CoA. (**d**) Typical LC-MS chromatogram of oxidized C20:4-CoA displaying mono- and di-oxygenated species. (**e**) Spectrum of mono- and di-oxygenated C20:4-CoA displaying singly and doubly negative charged ions. (**f**) Fragmentation spectrum and structure of di-oxygenated C20:4-CoA. (**g**) Fragmentation spectrum and structure of mono-oxygenated C20:4-CoA. (**h**) LC-MS chromatogram of mono-oxygenated arachidonic acid released after treatment of mono-oxygenated C20:4-CoA with hydroxylamine. (**i**) Spectrum of mono-oxygenated arachidonic acid released after treatment of mono-oxygenated C20:4-CoA with hydroxylamine.



Supplementary Figure 11. Identification of PEox molecular species in cell lysates. (**a**) Identification of PEox molecular species in lysates of Pfa1 cells supplemented with PE-(C18:0/C20:4) and treated with RSL3. Full MS spectra of PE and its oxygenated species formed in lysates of control and RSL3 treated Pfa1 cells after addition of PE-(C18:0/C20:4) plus 15-LOX or PE-(C18:0/C20:4-OOH) (left panels). MS spectra of oxygenated PE species with one, two and three oxygens detected in lysates of cells supplemented with PE-(C18:0/C20:4) and treated with RSL3 (right panels). (**b**) Identification of ferroptotic PEox molecular species in cell homogenates of Gpx4 KO cells. Full MS spectra of PE and its oxygenated species formed in cell lysates after addition of PE-(C18:0/C20:4) plus 15-LOX or PE-(C18:0/15-hydroperoxy-AA) (left panels). MS spectra of oxygenated PE species with one, two and three oxygens detected in Gpx4 KO cells (right panels). (**c**) MS/MS spectra of mono-oxygenated PE formed in PE-(C18:0/15-hydroperoxy-C20:4) treated Pfa1 cell lysates. Fragmentation pattern and structure of PE (18:0/15-hydroxy-C20:4).

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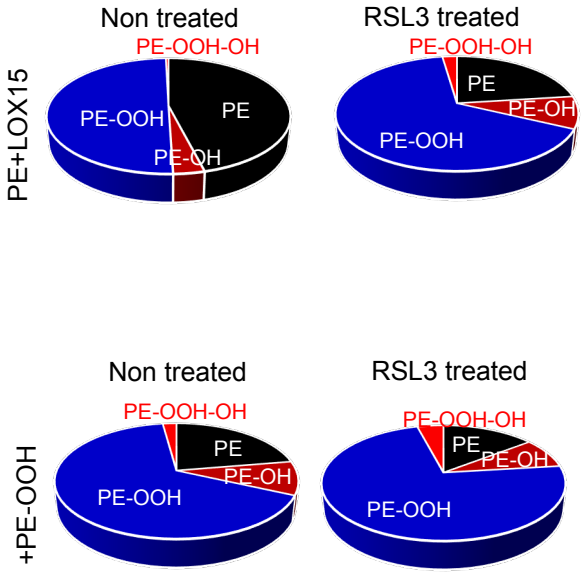
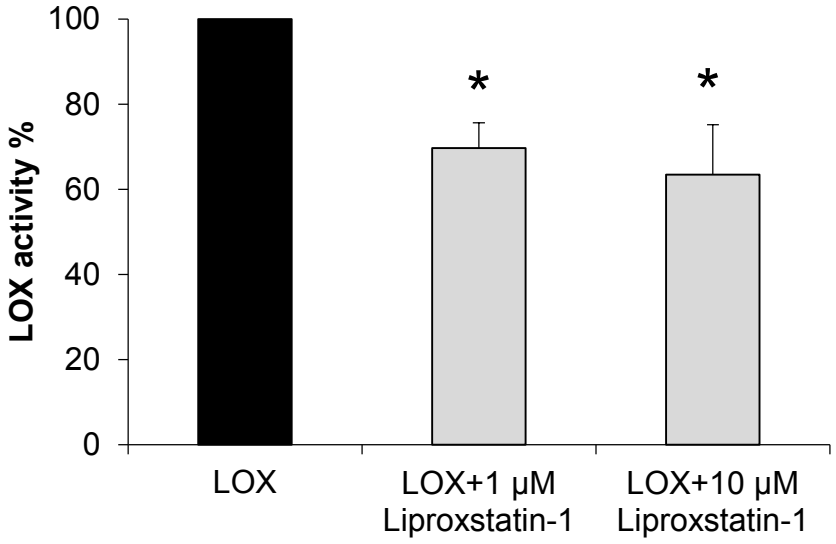


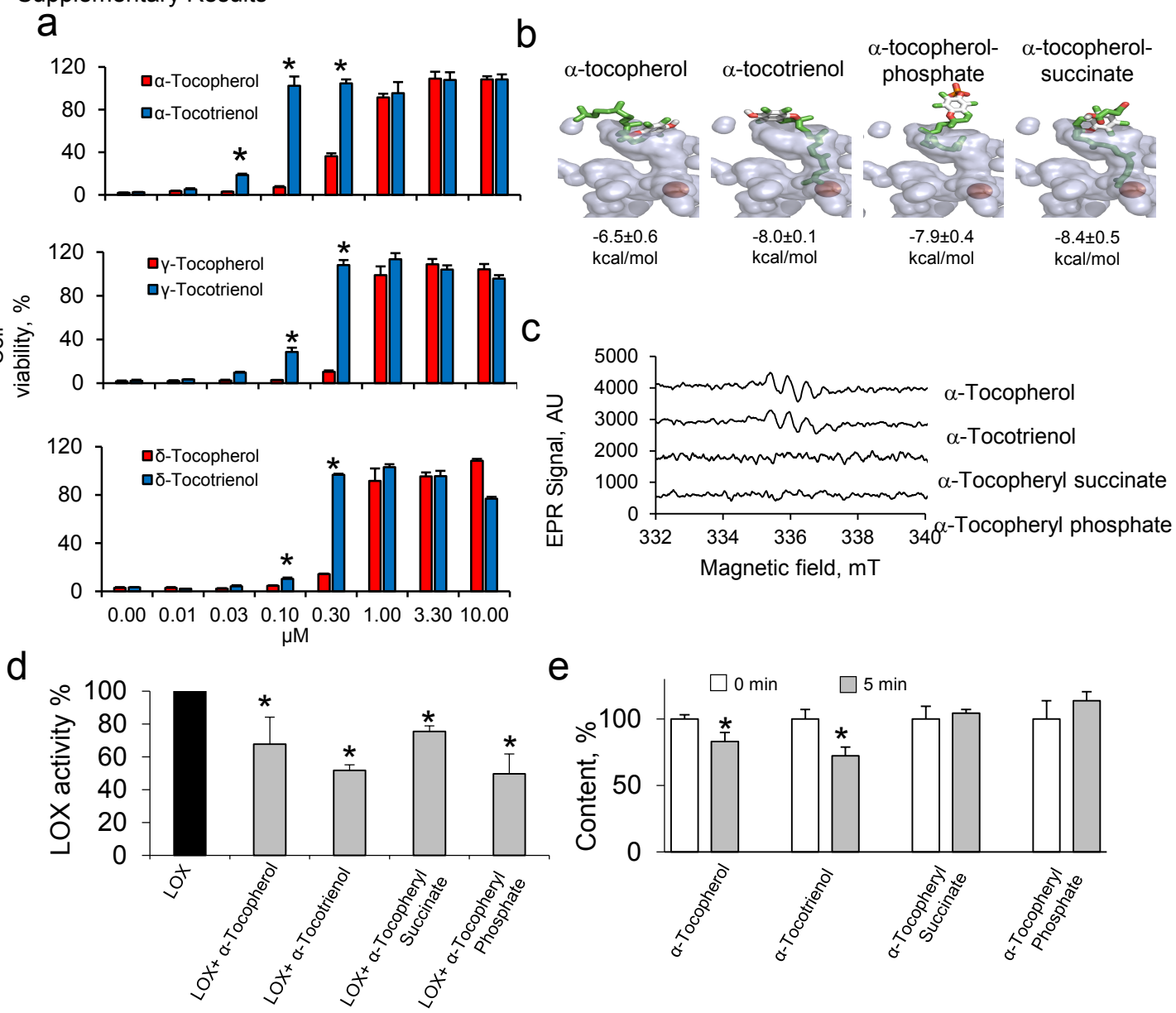
Supplementary Figure 12. Quantitative assessment of exogenous PE-(C18:0/C20:4) and its oxygenated products in Pfa1 cell lysates.



Supplementary Figure 13. 15-LOX activity in the presence of liproxstatin-1 in model system.

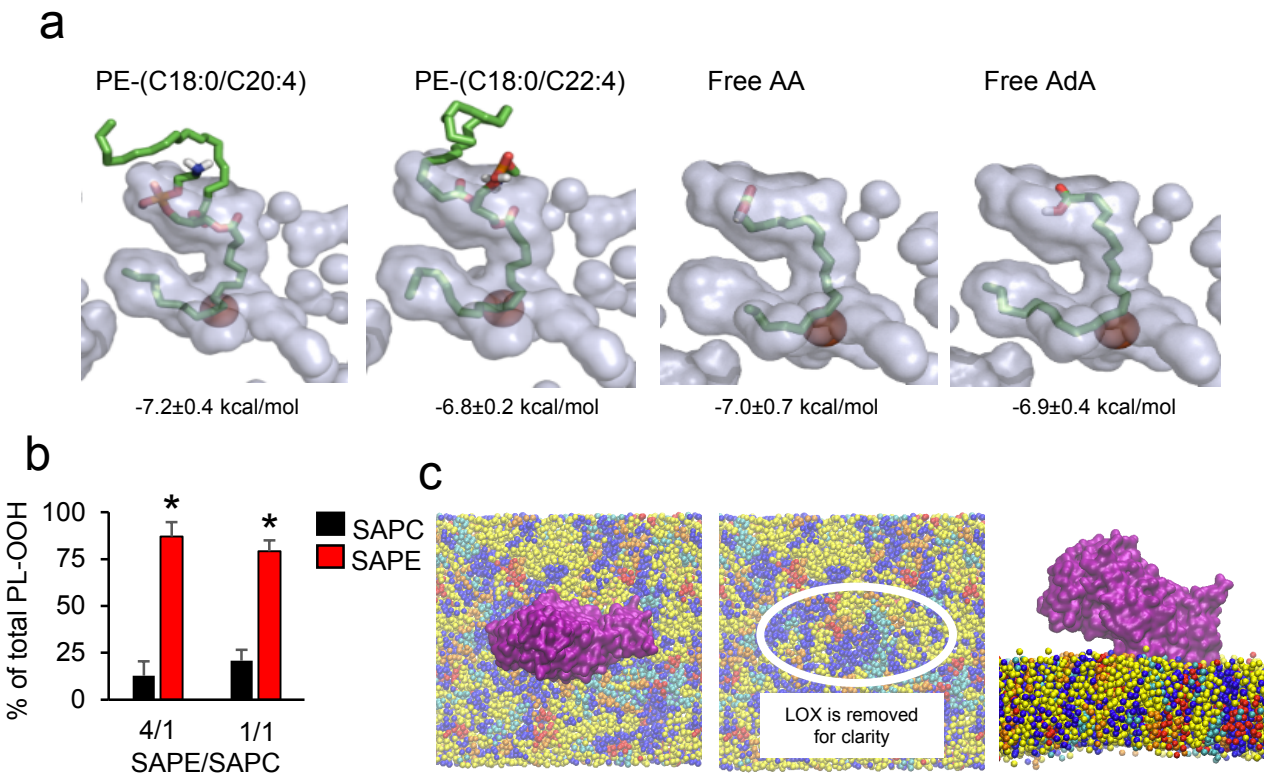
15-LOX (2.8 mU) was incubated with Liproxstatin-1 (1 or 10 μM) in 50 mM Tris-HCl buffer (pH 7.4) containing 20 μM AA, for 5 min at 37°C. The bar graphs represent % of LOX activity in each condition. Data are mean ± s.d., n=3. *P < 0.05 by t-test vs. samples containing only LOX15 (black bar).

Supplementary Results



Supplementary figure 14. Interactions of the enzyme with tocotrienols and tocopherols, protective effects of these vitamin E homologues against ferroptosis and Effects of non-esterified (α-tocopherol or α-tocotrienol) and esterified forms of tocopherols (α-tocopherol succinate and α-tocopherol phosphate) on the 15-LOX activity.

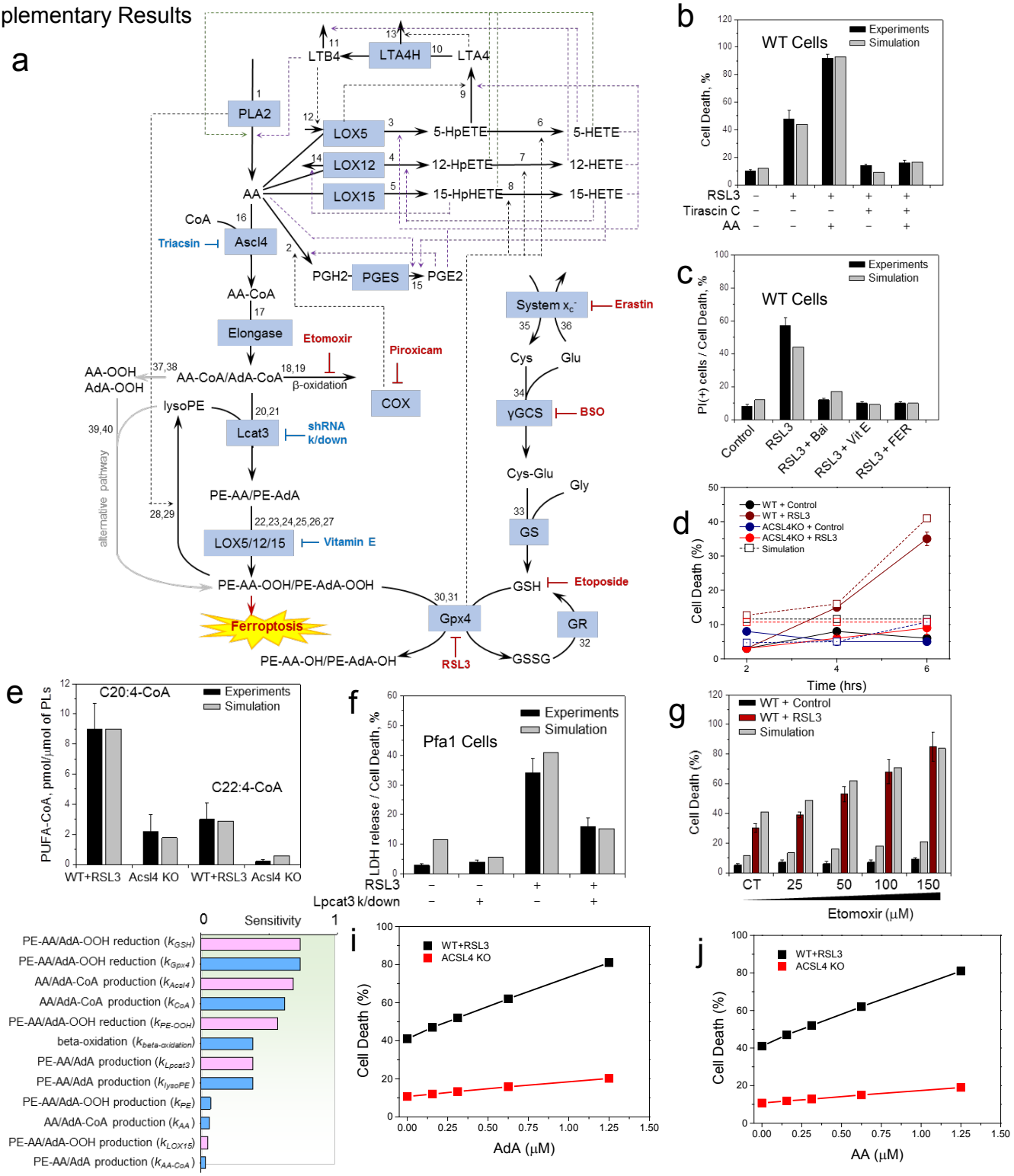
(**a**) Tocopherols and tocotrienols – are effective protectors against ferroptosis in Gpx4 KO cells. Data are mean ± s.d., n=3. *P < 0.05 by t-test v.s. tocopherol treated cells. Cell viability was assessed by AquaBluer. (**b**) Molecular docking modeling of binding of 15-LOX-2 to vitamin E homologues and two α-tocopherol esterified derivatives. (**c**) ESR spectra of phenoxyl radicals generated in the reaction system containing 15-LOX/AA in the presence of α-tocopherol, α-tocotrienol, tocopheryl succinate and tocopheryl phosphate. (**d**) 15-LOX activity in the presence of α-tocopherol, α-tocotrienol, α-tocopheryl succinate and α-tocopheryl phosphate. All assays were performed at 37°C in 50 mM Tris-HCl buffer (pH 7.4) containing 20 μM AA, and 2.8 mU 15-LOX. Each vitamin E is 10 μM. Data are mean ± s.d., n=3. *P < 0.05 by t-test v.s. samples containing only LOX15 (black bar). (**e**) Content of α-tocopherol, α-tocotrienol, α-tocopheryl succinate and α-tocopheryl phosphate after incubation with 15-LOX and AA in 50 mM TrisHCL buffer pH 7.4 at 37°C. Open bars, 0 min, Closed bars, 5 min. The contents of α-tocopherol and α-tocotrienol were reduced by 85 ±34 and 138±33 pmol/sample, (decreased by 17% and 28%), respectively. Under the same incubation conditions, 15-LOX oxidized 380±17 pmol of AA per sample in the absence of α-tocopherol or α-tocotrienol. Data are mean ± s.d., n=3. *P < 0.05 by t-test v.s. each 0 minute sample.



Supplementary figure 15. Characterization of lipoxygenase-catalyzed oxidation of PUFA-phospholipids

(**a**) Molecular docking shows that PE-(C18:0/C20:4) and PE-(C18:0/C22:4) bind to 15-LOX-2 active site similarly with binding energies of -7.2 ± 0.4 and -6.8 ± 0.2, respectively, n=3 – compare with binding of free AA and free AdA -7.0 ± 0.7 and -6.9 ± 0.4, respectively. (**b**) Human recombinant 15-LOX-2 is more effective in oxidizing SAPE (PE-C18:0/C20:4) than SAPC (PC-C18:0/C20:4) in model system. Data are mean ± s.d., n=3. *P < 0.05 by t-test v.s. control. **(c)** Coarse-grained molecular dynamics simulations of interactions of human 15-LOX with a membrane containing three species of PC (16:0/16:0; p18:1/18:2; 18:1/20:4) and two species of PE (SAPE and alkenyl-SAPE) show that PC displayed a poor capacity for binding 15-LOX as compared to PE. Left panel. Top views of a typical representation of 15LOX-2 interacting with the membrane. Middle panel. Top view — 15LOX-2 has been removed for clarity. Right panel. Side view. The majority of PE (~80%) is confined to the inner (cytoplasmic) leaflet of plasma membrane thus permitting direct interactions with the oxidizing intracellular mechanisms, including LOX. In contrast, phosphatidylcholines and sphingomyelins are dominant phospholipids in the outer leaflet exposed to extracellular compartments. This asymmetric distribution can make PE a more favorable substrate for LOX. Representation guide: All beads representing a lipid are shown in one color; yellow: PC (16:0/16:0), orange: alkenyl-PC (pPC) (18:1p/18:2), red: PC (18:0/20:4), blue: PE (18:0/20:4), cyan: alkenyl-PE (pPE) (18:0p/20:4). 15LOX-2 is illustrated in purple surface representation.

Supplementary Results



Supplementary Figure 16. Systems biology analysis of metabolic networks participating in the generation of ferroptotic oxygenated PE signals in Gpx4 deficient cells.

(**a**) Detailed reaction scheme for the metabolic network model. (**b-e**) Comparison of model predictions and the experimental data sets used for training model parameters. Experimental data are mean ± s.d., n=3. (**f-g**) Comparison of model predictions and the experimental data sets used reserved for model validation. Experimental data are mean ± s.d., n=3. (**h**) Control coefficient-based sensitivities of the kinetic parameters of reactions (with sensitivity > 0.01) to the integrated response of ferroptotic cell death. (**i-j**) Model predicted ferroptotic cell death in response to exogenous AdA and AA in WT cells treated with RSL3 and Acsl4 KO cells, indicating dose dependence of ferroptosis on exogenous AA/AdA.

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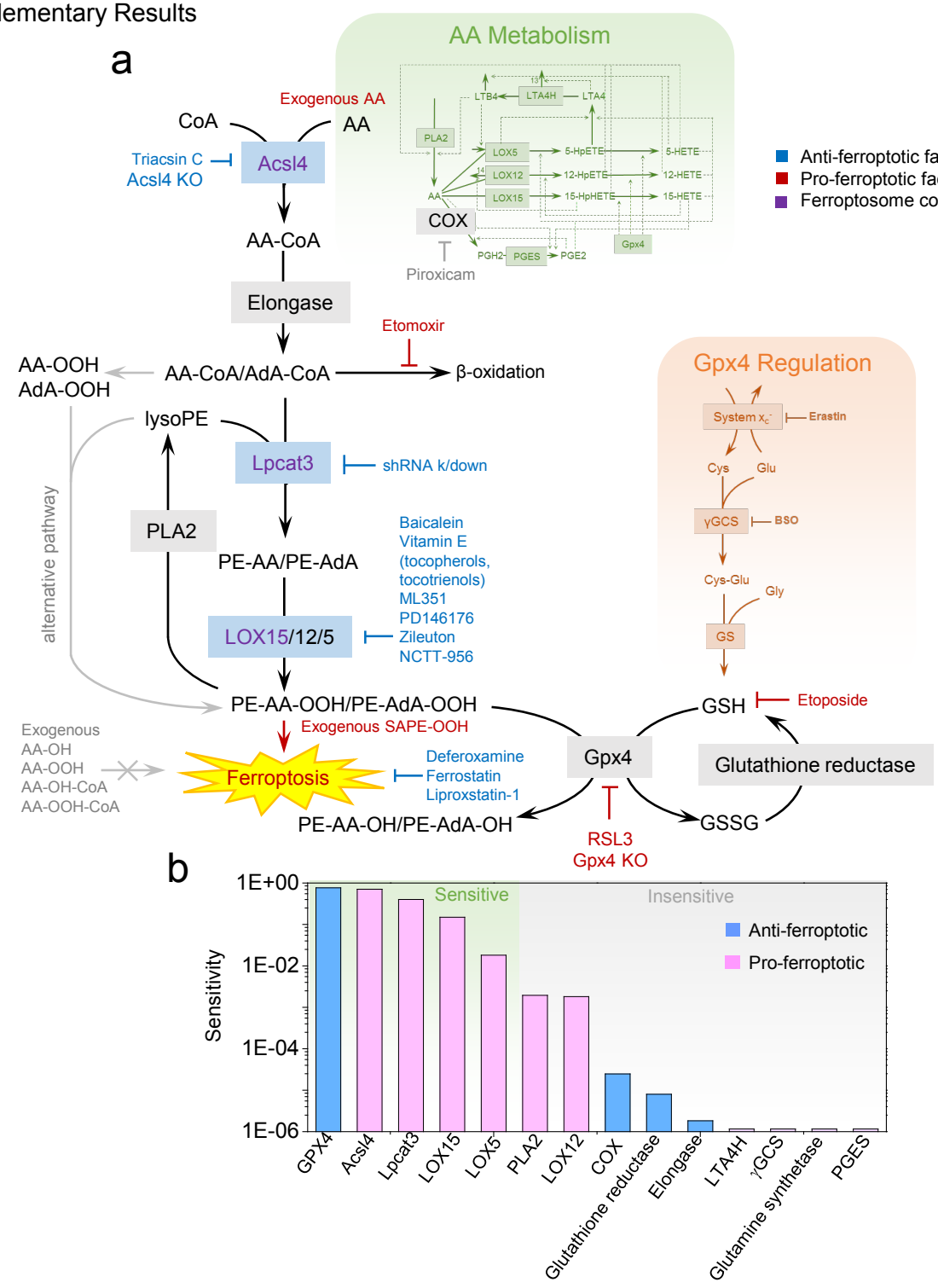
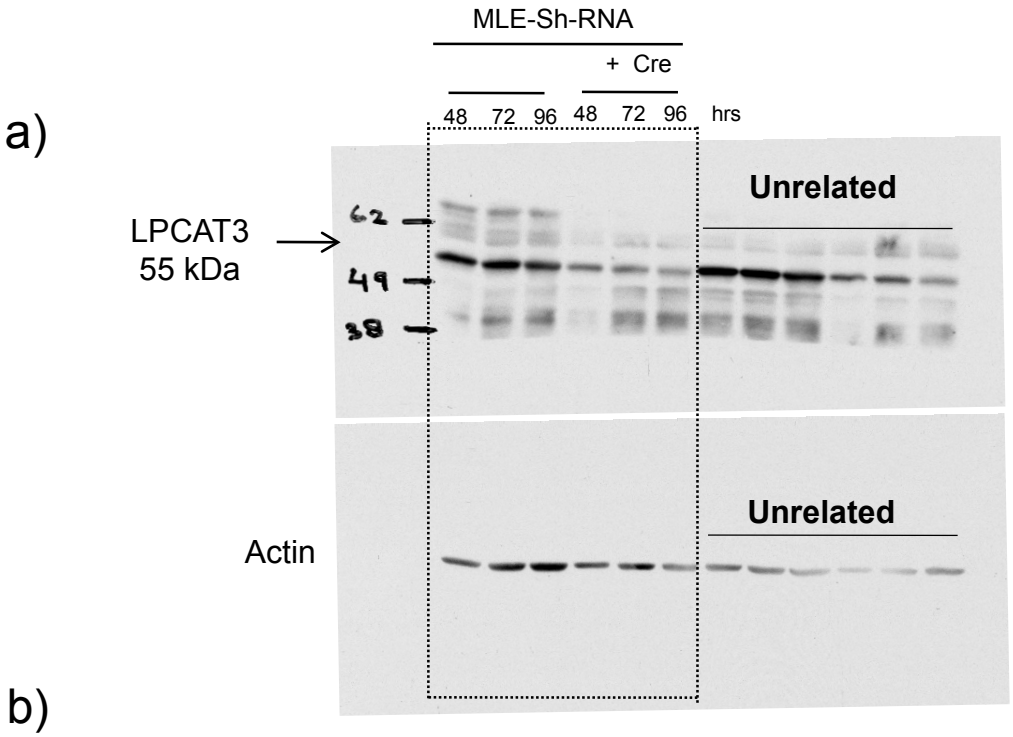
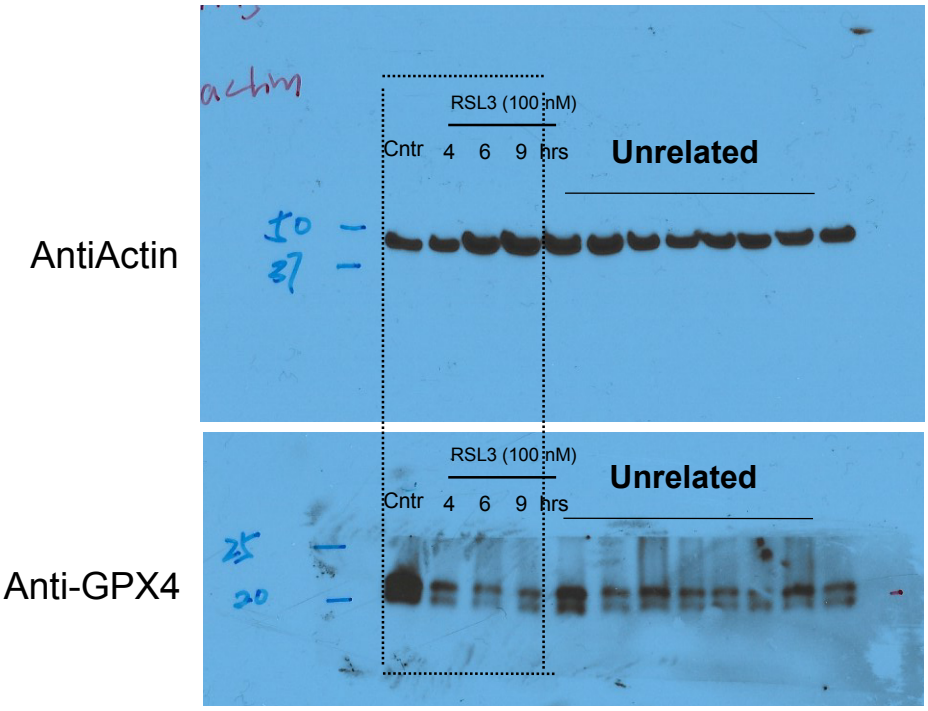


Supplementary Figure 17. Computational modeling of phospholipid metabolic network regulating ferroptosis.

(a) AA-initiated metabolic and Gpx4-dependent regulation of oxygenated PE ferroptotic signals. Single-headed solid arrows designate irreversible reactions. Dotted arrows represent enzymatic reactions. Arrows with bar-heads represent inhibition. The AA metabolism and Gpx4 regulation modules are presented as abstract blocks. Detailed reaction schema and kinetic equations are presented in Supplementary table 4. **(b)** Control coefficient-based sensitivities of the initial concentrations of enzymes to the integrated ferroptotic death. The threshold sensitivity for classifying sensitive or insensitive enzymes is 0.01. The model is in quantitative agreement with the following systems-level insights: (i) identification of oxygenated PE species as a prognostic in silico platform; (ii) sensitivity analysis-confirmed key regulators of ferroptosis: Gpx4 and Acsl4 as the major anti- and pro-ferroptotic factors respectively, as well as 15-LOX as the generator of oxygenated PE-AA/AdA.



RSL3 treated MEF cells:



Supplementary Figure 18: Full western blots used for a) **Figure 2f** and b) **Supplementary Figure 1c.**