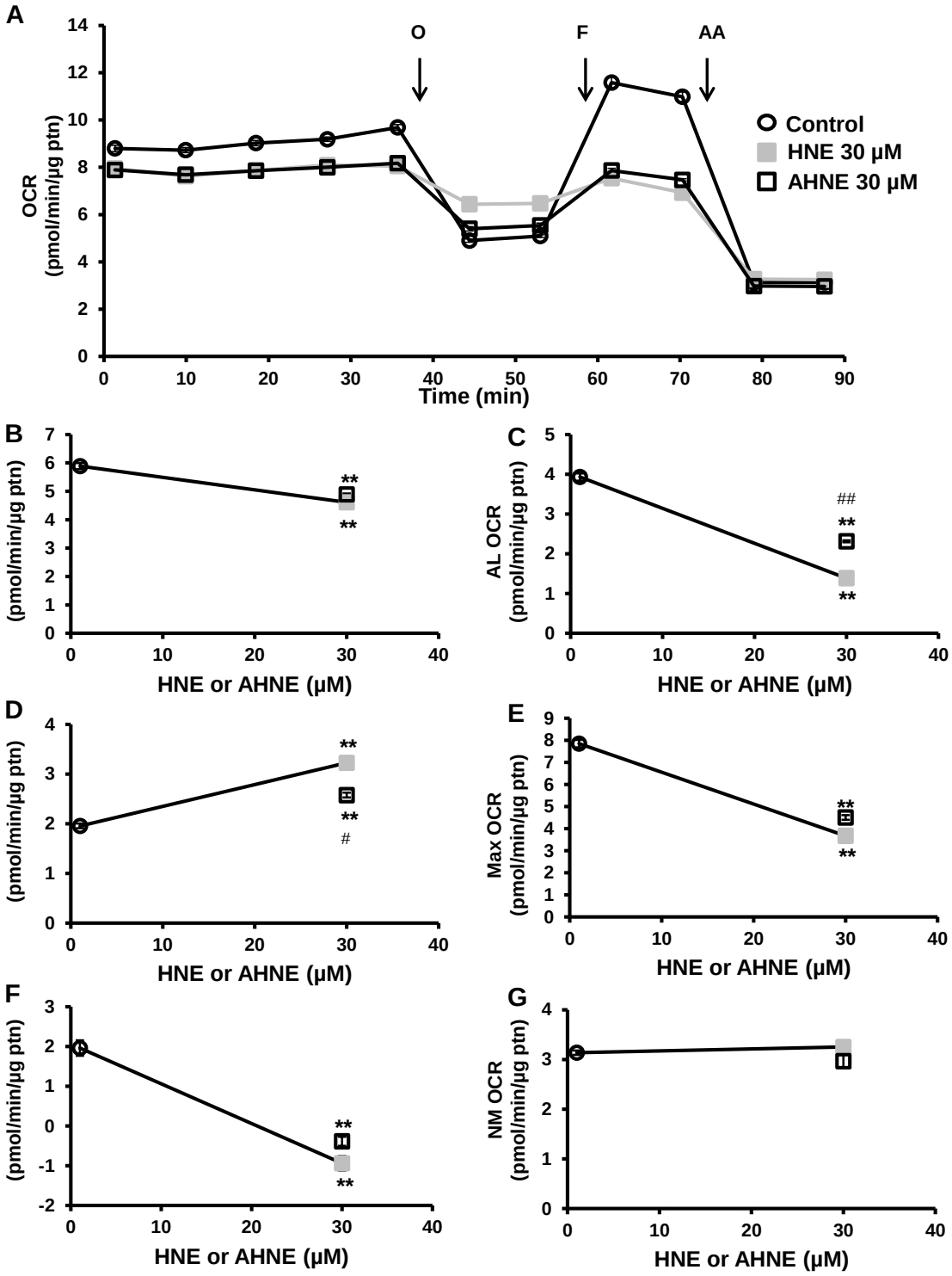
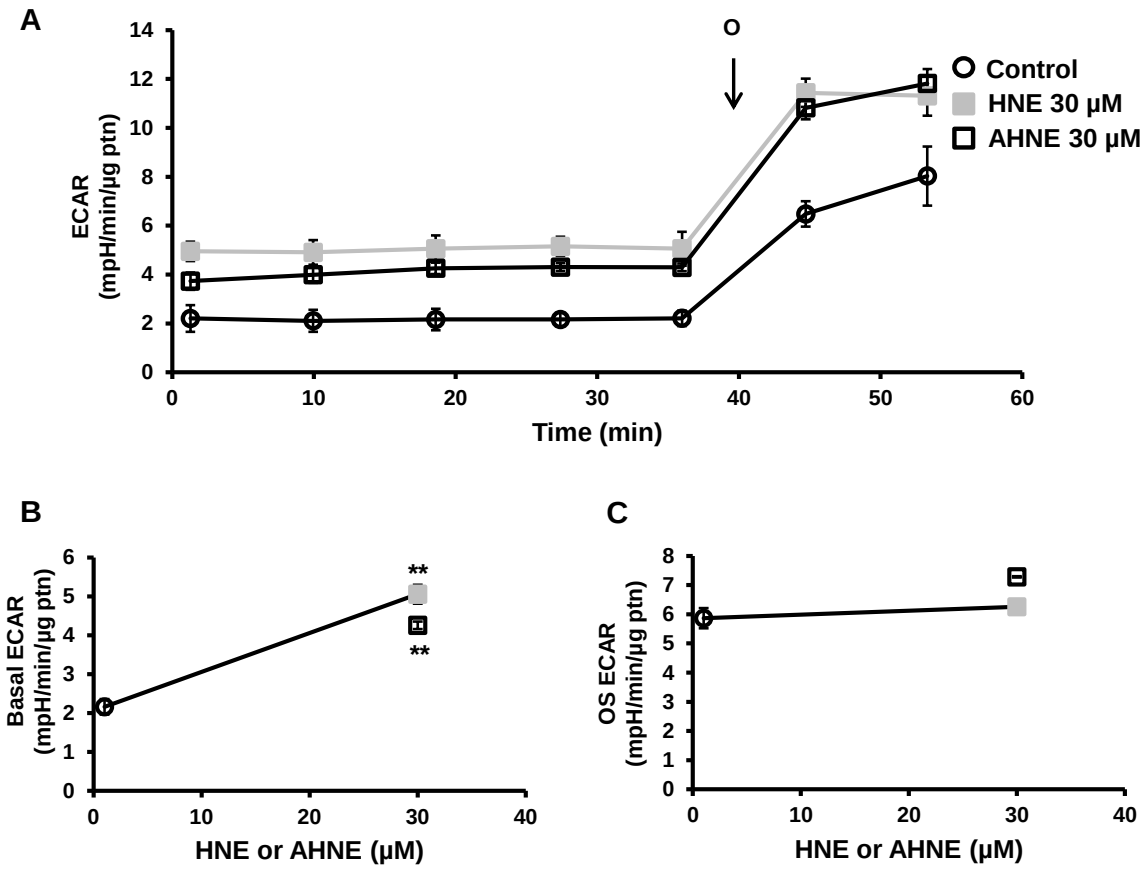
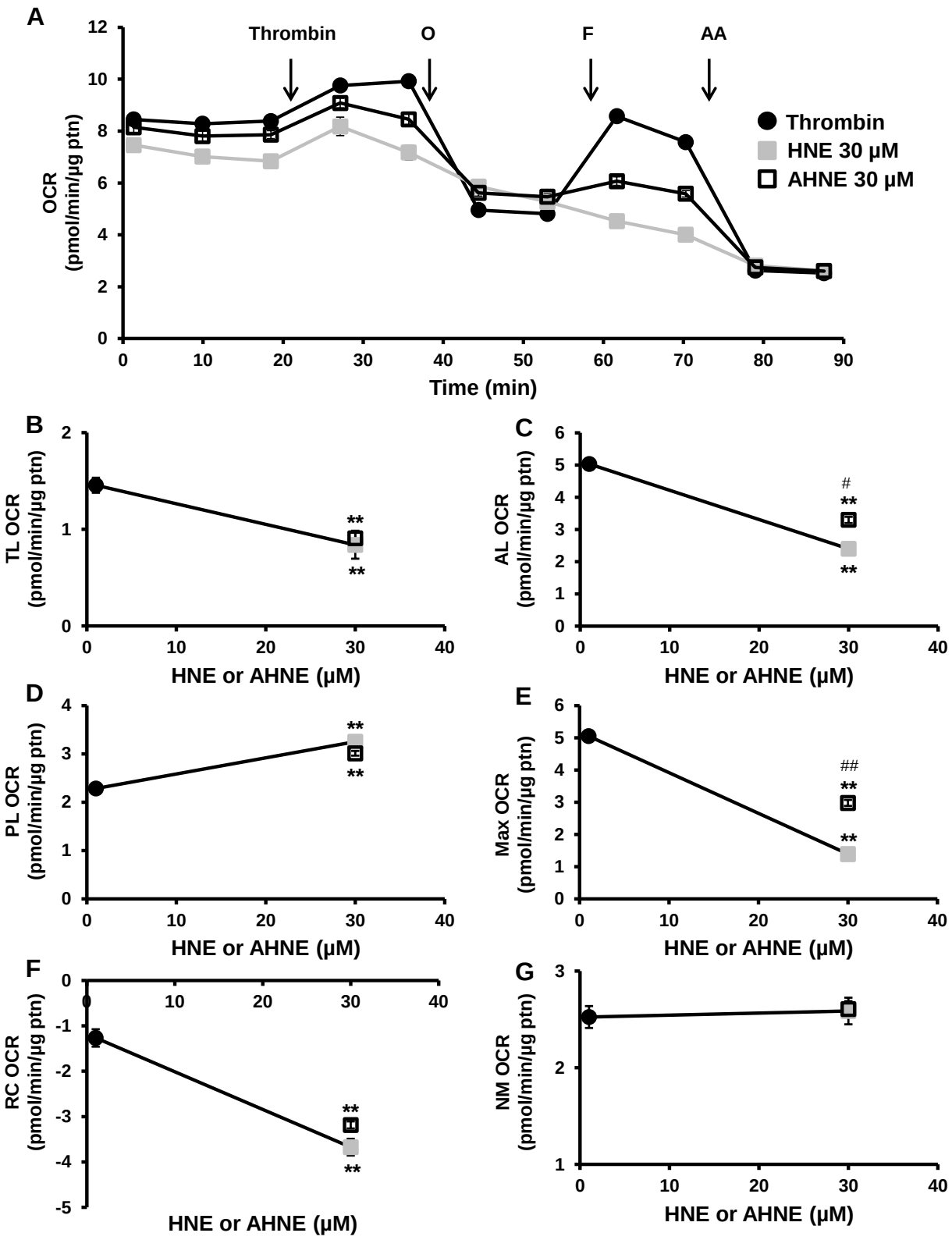
Supplementary Data



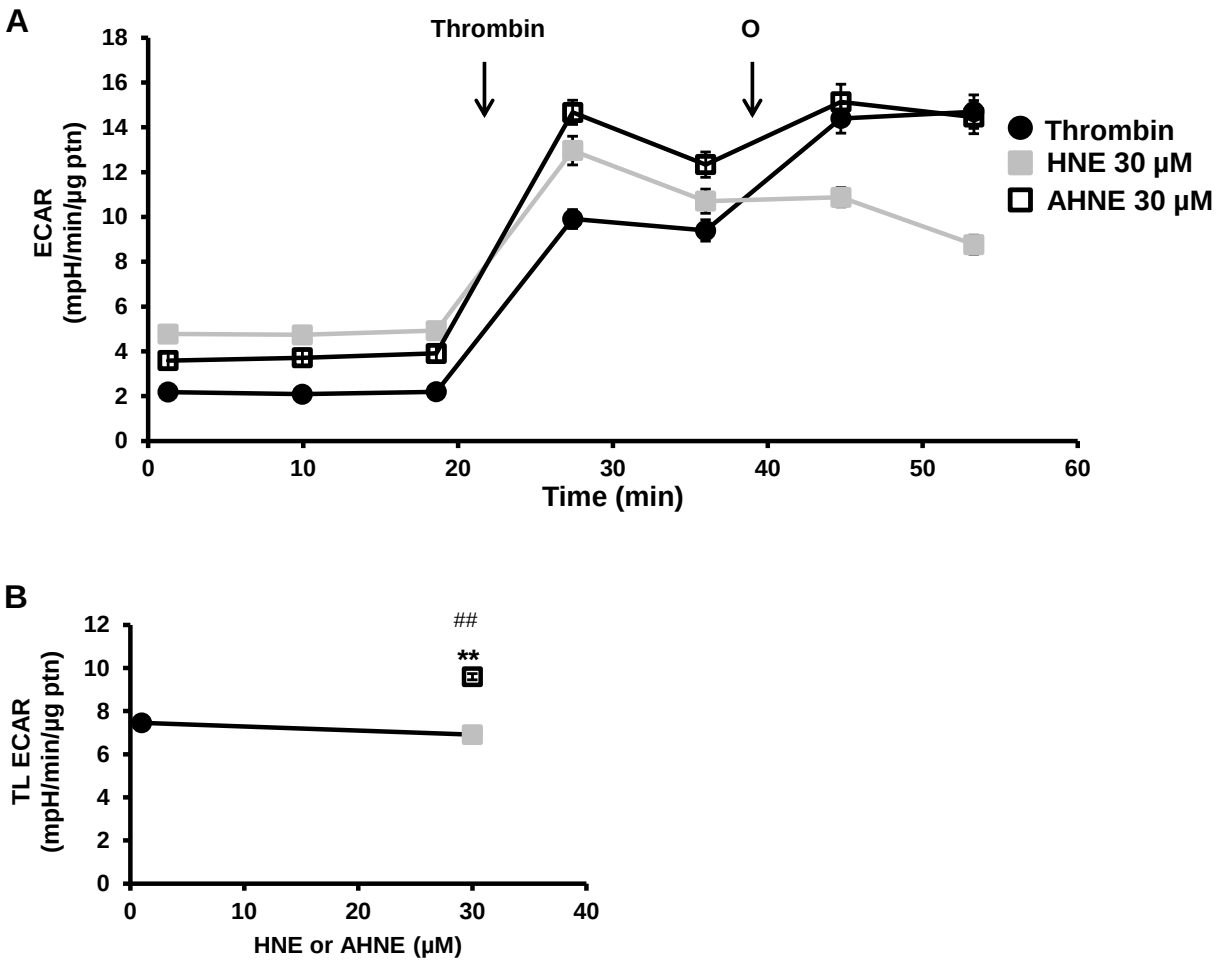
Supplementary Figure 1: Comparing the effects of A-HNE to 4-HNE on mitochondrial respiration. (A) Platelets were incubated with AHNE (30 µM) or 4-HNE (HNE) (0-30 µM) for 1h after which a mitochondrial stress test was performed by first measuring basal OCR, followed by sequential injection of oligomycin (O) (1 µg/ml), FCCP (F) (0.6 µM), antimycin A (AA) (10 µM). Different indices of mitochondrial respiration (B) basal (basal OCR – AA OCR), (C) ATP-linked (AL) (basal OCR – oligomycin OCR), (D) proton leak (PL) (oligomycin OCR – AA OCR), (E) maximal (FCCP OCR – AA OCR), (F) reserve capacity (RC) (FCCP OCR – basal OCR) and (G) non-mitochondrial (NM) (AA OCR) were calculated. Data expressed as mean±SEM from one representative donor, n = 5-6 replicates. **p<0.01, different from thrombin. #p<0.05, ##p<0.01, different from 4-HNE (30 µM).



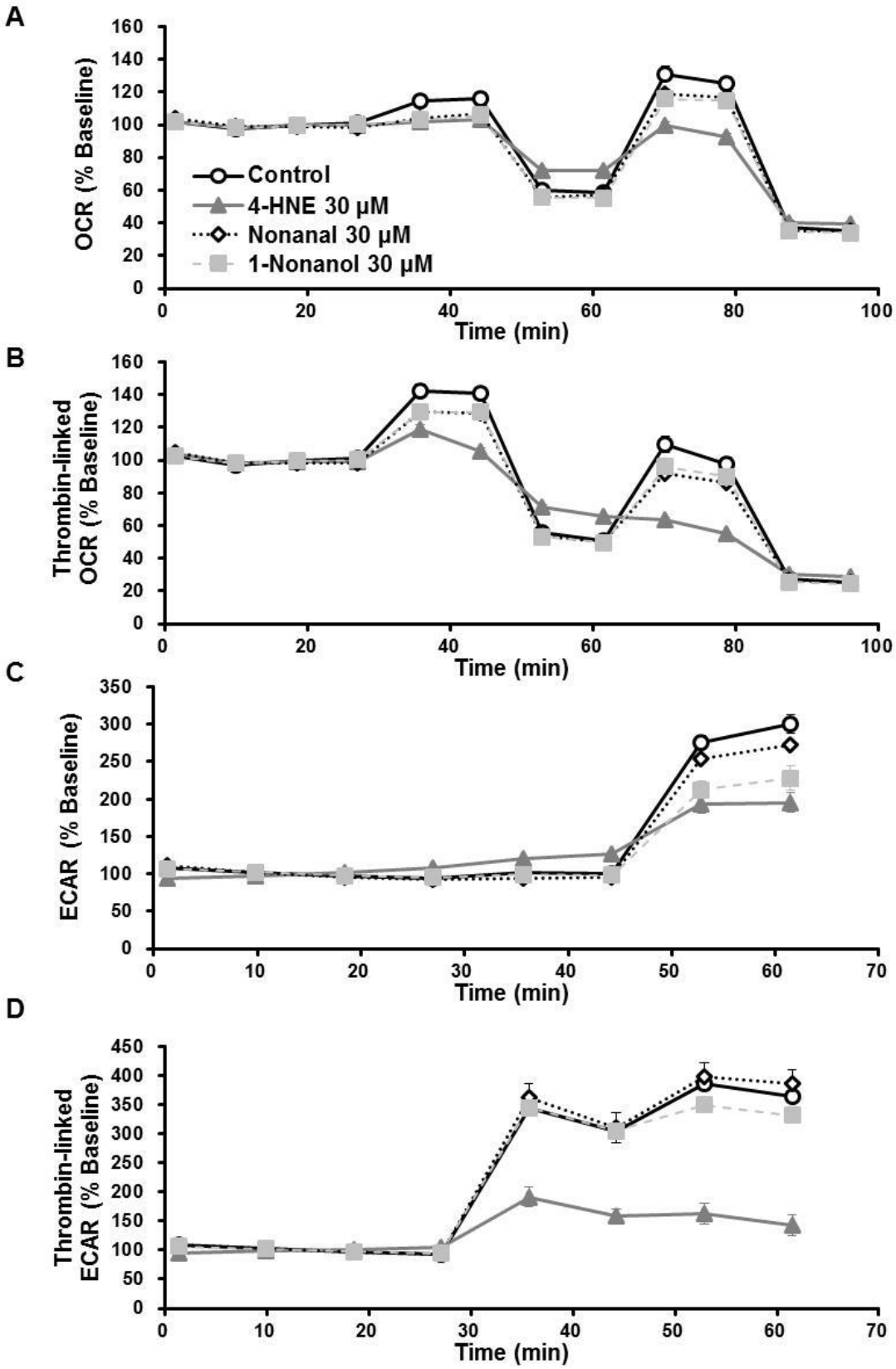
Supplementary Figure 2: Comparing the effects of A-HNE to 4-HNE on glycolysis. (A) Platelets were incubated with AHNE (30 µM) or 4-HNE (HNE) (0-30 µM) for 1h after which basal ECAR was measured, followed by injection of oligomycin (O) (1 µg/ml). Different indices of glycolytic function (B) basal (basal ECAR) and (C) oligomycin sensitive (OS) (oligomycin ECAR – basal ECAR) were calculated. Data expressed as mean±SEM from one representative donor, n = 5-6 replicates. ***p<0.01, different from thrombin. ##p<0.01, different from 4-HNE (30 µM).


A
Thrombin
O
F
AA
Thrombin
HNE 30 μM
AHNE 30 μM
OCR (pmol/min/μg ptn)
Time (min)
B
TL OCR (pmol/min/μg ptn)
HNE or AHNE (μM)
C
AL OCR (pmol/min/μg ptn)
HNE or AHNE (μM)
D
PL OCR (pmol/min/μg ptn)
HNE or AHNE (μM)
E
Max OCR (pmol/min/μg ptn)
HNE or AHNE (μM)
F
RC OCR (pmol/min/μg ptn)
HNE or AHNE (μM)
G
NM OCR (pmol/min/μg ptn)
HNE or AHNE (μM)

Supplementary Figure 3: Comparing the effect of A-HNE to 4-HNE on mitochondrial respiration in the presence of thrombin. (A) Platelets were incubated with AHNE (30 µM) or 4-HNE (HNE) (0-30 µM) for 1h after which a mitochondrial stress test was performed by first measuring basal OCR, followed by sequential injection of thrombin (0.5 U/ml) oligomycin (O) (1 µg/ml), FCCP (F) (0.6 µM), antimycin A (AA) (10 µM). Different indices of mitochondrial respiration (B) thrombin linked (TL) (thrombin OCR – basal OCR), (C) ATP-linked (AL) (thrombin OCR – oligomycin OCR), (D) proton leak (PL) (oligomycin OCR – AA OCR), (E) maximal (FCCP OCR – AA OCR), (F) reserve capacity (RC) (FCCP OCR – thrombin OCR) and (G) non-mitochondrial (NM) (AA OCR) were calculated. Data expressed as mean±SEM from one representative donor, n = 5-6 replicates. **p<0.01, different from thrombin. #p<0.05, ##p<0.01, different from 4-HNE (30 µM).



Supplementary Figure 4: Comparing the effect of A-HNE to 4-HNE on glycolysis in the presence of thrombin. (A) Platelets were incubated with AHNE (30 µM) or 4-HNE (HNE) (0-30 µM) for 1h after which basal ECAR was measured, followed by sequential injections of thrombin (0.5 U/ml) and oligomycin (O) (1 µg/ml). Different indices of glycolytic function (B) thrombin linked (TL) (thrombin ECAR) and (C) oligomycin sensitive (OS) (oligomycin ECAR – thrombin ECAR) were calculated. Data expressed as mean±SEM from one representative donor, n = 5-6 replicates. **p<0.01, different from thrombin. ##p<0.01, different from 4-HNE (30 µM).


A
OCR (% Baseline)
Control
4-HNE 30 μM
Nonanal 30 μM
1-Nonanol 30 μM
Time (min)
B
Thrombin-linked OCR (% Baseline)
Time (min)
C
ECAR (% Baseline)
Time (min)
D
Thrombin-linked ECAR (% Baseline)
Time (min)

Supplementary Figure 5: Comparing the effect of 4-HNE to its non-electrophilic analogs, nonanal and 1-nonanol in the presence and absence of thrombin. Platelets were incubated with either vehicle control, HNE, nonanal or 1-nonanol (all 30 μM) for 1h, after which basal OCR (A) was measured, following by thrombin-stimulated OCR (B). Glycolytic function (C) and thrombin-linked (TL) (thrombin ECAR) (D) were also measured. Data expressed as mean ± SEM from two representative donors.

N	Name	Accession	Peptides	%Cov
1	Integrin alpha-6	P23229	2	1
2	Multimerin-1	Q13201	3	3
3	Platelet glycoprotein IX	P14770	2	12

Supplementary Table 1: A-HNE modification of adhesion related proteins. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	Actin-related protein 2/3 complex subunit 2	O15144	2	7
2	Actin-related protein 2/3 complex subunit 3	O15145	3	16
3	Actin-related protein 2/3 complex subunit 4	P59998	5	30
4	Actin-related protein 3	P61158	5	11
5	Adenylyl cyclase-associated protein 1	Q01518	7	12
6	Bridging integrator 2	Q9UBW5	5	9
7	Coronin-1C	Q9ULV4	3	6
8	F-actin-capping protein subunit alpha-2	P47755	2	6
9	F-actin-capping protein subunit beta	P47756	3	14
10	Myosin regulatory light chain 12A	P19105	5	24
11	Myosin regulatory light chain 12B	O14950	5	24
12	Myosin regulatory light polypeptide 9	P24844	4	17
13	Myosin-10	P35580	6	3
14	Putative tropomyosin alpha-3 chain-like protein	A6NL28	6	17
15	Substrate adhesion	Q14247	6	7
16	Tropomyosin alpha-3 chain	P06753	10	31
17	Tropomyosin alpha-4 chain	P67936	18	55
18	Tubulin alpha-8 chain	Q9NY65	9	16
19	Vasodilator-stimulated phosphoprotein	P50552	4	12
20	Zyxin	Q15942	8	20

Supplementary Table 2: A-HNE modification of proteins involved in cytoskeletal reorganization. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	Calnexin	P27824	2	3
2	Endoplasmin	P14625	7	9
3	Heat shock protein HSP 90-alpha	P07900	10	13
4	Heat shock protein HSP 90-beta	P08238	6	9
5	Peptidyl-prolyl cis-trans isomerase F, mitochondrial	P30405	2	8
6	60 kDa heat shock protein, mitochondrial	P10809	2	3

Supplementary Table 3: A-HNE modifications of proteins involved in protein folding. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	GTP-binding protein SAR1a	Q9NR31	2	12
2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	P62873	2	6
3	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	P62879	2	6
4	Guanine nucleotide-binding protein subunit beta-4	Q9HAV0	2	6
5	Rab GDP dissociation inhibitor alpha	P31150	2	4
6	Rab GDP dissociation inhibitor beta	P50395	2	4
7	Ras-related protein Rab-11A	P62491	3	13
8	Ras-related protein Rab-11B	Q15907	3	13
9	Ras-related protein Rab-27B	O00194	3	9
10	Ras-related protein Rab-6A	P20340	2	11
11	Ras-related protein Rab-6B	Q9NRW1	2	11

Supplementary Table 4: A-HNE modification of small GTPase proteins. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	ATP synthase subunit alpha, mitochondrial	P25705	5	11
2	ATP synthase subunit beta, mitochondrial	P06576	7	18
3	Hexokinase-1	P19367	5	5
4	Isocitrate dehydrogenase [NADP], mitochondrial	P48735	7	17
5	Malate dehydrogenase, cytoplasmic	P40925	2	6
6	Malate dehydrogenase, mitochondrial	P40926	4	12
7	Solute carrier family 2, facilitated glucose transporter member 14	Q8TDB8	2	3
8	Solute carrier family 2, facilitated glucose transporter member 3	P11169	2	3
9	Triosephosphate isomerase	P60174	4	21
10	Voltage-dependent anion-selective channel protein 3	Q9Y277	2	7

Supplementary Table 5: A-HNE modification of metabolism related proteins. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	Integrin beta-3	P05106	13	14
2	Leukocyte elastase inhibitor	P30740	3	8
3	Serum albumin	P02768	6	8

Supplementary Table 6: A-HNE modification of proteins involved in aggregation. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	Glutathione peroxidase 1	P07203	2	11
2	Glutathione S-transferase P	P09211	2	15
3	Peroxiredoxin-1	Q06830	2	11
4	Peroxiredoxin-6	P30041	2	9
5	Superoxide dismutase [Mn], mitochondrial	P04179	3	14

Supplementary Table 7: A-HNE modification of antioxidant enzymes. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	Calpain-1 catalytic subunit	P07384	6	6
2	Carbonic anhydrase 2	P00918	3	11
3	Chloride intracellular channel protein 1	O00299	6	28
4	Galectin-related protein	Q3ZCW2	3	19
5	Protein disulfide-isomerase	P07237	3	6
6	Protein disulfide-isomerase A3	P30101	11	21
7	Protein S100-A8	P05109	3	31
8	Protein S100-A9	P06702	3	31
9	Proto-oncogene tyrosine-protein kinase Src	P12931	4	6
10	Purine nucleoside phosphorylase	P00491	6	19
11	Syntaxin-binding protein 2	Q15833	2	3

Supplementary Table 8: A-HNE modification of proteins involved in signaling activities. List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.

N	Name	Accession	Peptides	%Cov
1	EH domain-containing protein 3	Q9NZN3	2	4
2	Reticulon-4	Q9NQC3	2	2
3	Transitional endoplasmic reticulum ATPase	P55072	4	5

Supplementary Table 9: A-HNE modifications on proteins involved vesicular transport. *List of proteins identified in the A-HNE treated platelets, with protein accession ID, number of peptides identified with 95% confidence, percent coverage of the peptides identified to the sequence of the protein with 95% confidence.*