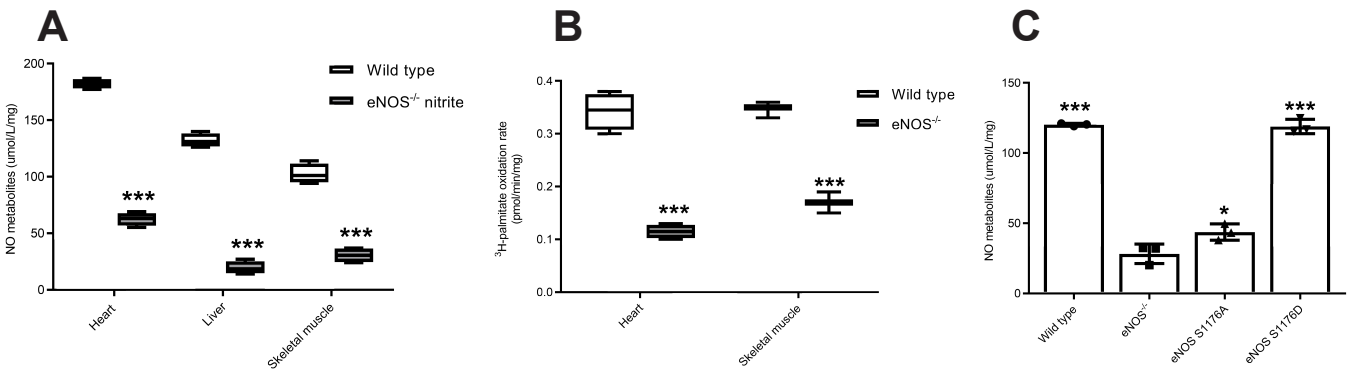
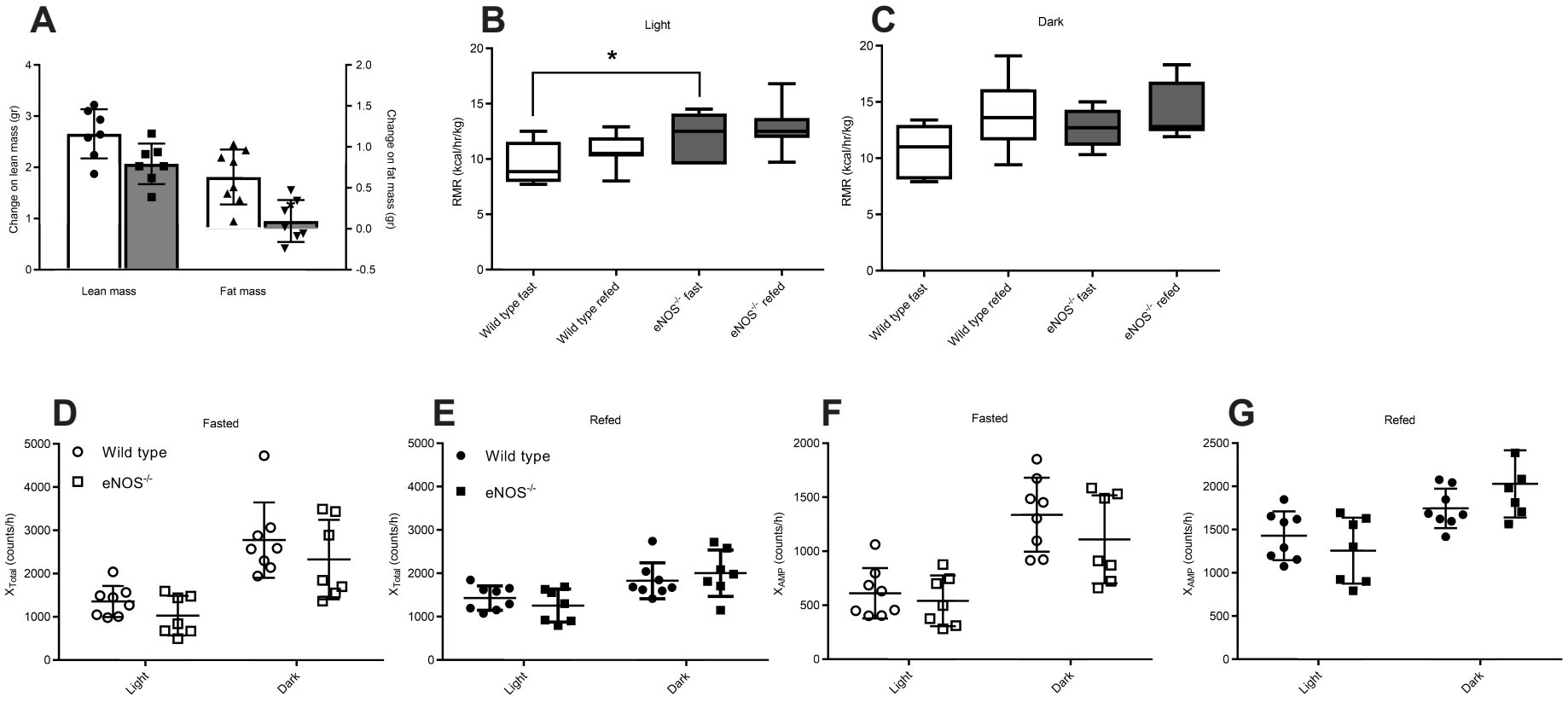
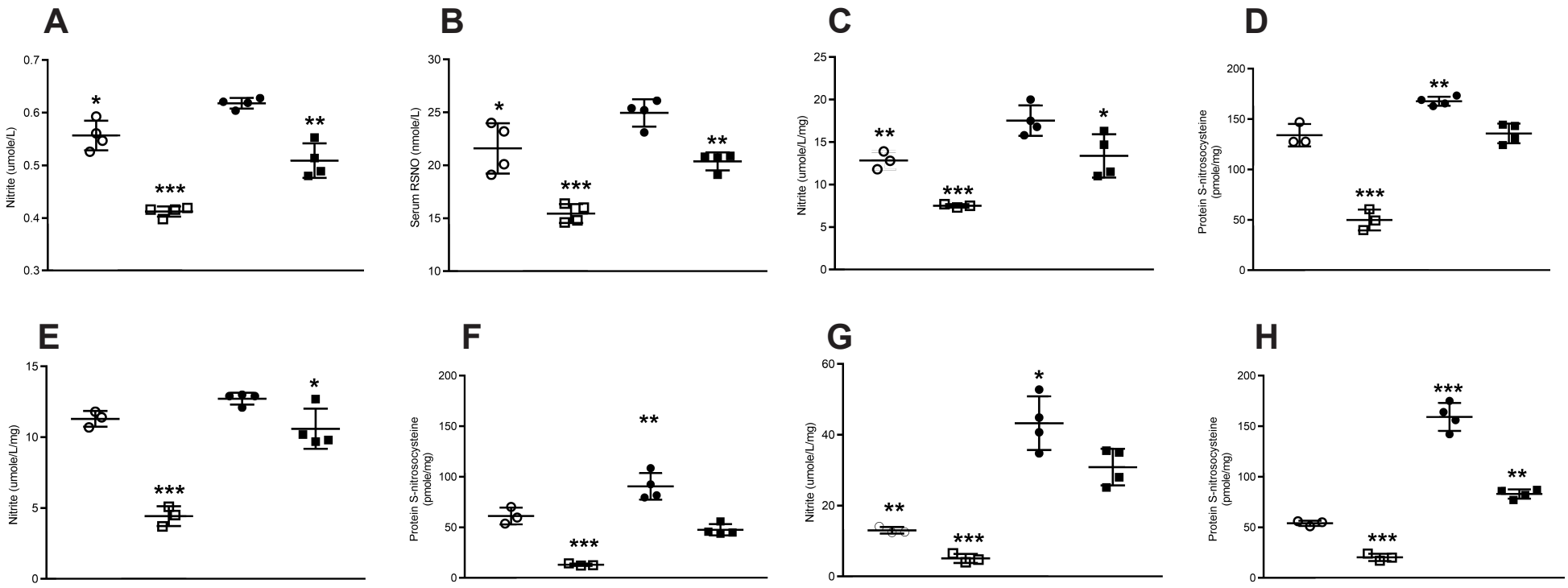

A
B
C
Wild type
eNOS-/- nitrite
NO metabolites (umol/L/mg)
200
150
100
50
0
***
***
***
Heart
Liver
Skeletal muscle
Wild type
eNOS-/-
3H-palmitate oxidation rate (pmol/min/mg)
0.4
0.3
0.2
0.1
0.0
***
***
Heart
Skeletal muscle
NO metabolites (umol/L/mg)
150
100
50
0
***
*
***
Wild type
eNOS-/-
eNOS S1176A
eNOS S1176D


A
Change on lean mass (gr)
Change on fat mass (gr)
Lean mass
Fat mass
B
Light
RMR (kcal/hr/kg)
Wild type fast
Wild type refed
eNOS-/- fast
eNOS-/- refed
C
Dark
RMR (kcal/hr/kg)
Wild type fast
Wild type refed
eNOS-/- fast
eNOS-/- refed
D
Fasted
Wild type
eNOS-/-
XTotal (counts/h)
Light
Dark
E
Refed
Wild type
eNOS-/-
XTotal (counts/h)
Light
Dark
F
Fasted
XAMP (counts/h)
Light
Dark
G
Refed
XAMP (counts/h)
Light
Dark


A
Nitrite (umole/L)
0.7
0.6
0.5
0.4
0.3
*
***
**
B
Serum RSNO (nmole/L)
30
25
20
15
10
*
***
**
C
Nitrite (umole/L/mg)
25
20
15
10
5
0
**
***
*
D
Protein S-nitrosocysteine (pmole/mg)
200
150
100
50
0
**
***
E
Nitrite (umole/L/mg)
15
10
5
0
***
*
F
Protein S-nitrosocysteine (pmole/mg)
200
150
100
50
0
***
**
G
Nitrite (umole/L/mg)
60
40
20
0
**
***
*
H
Protein S-nitrosocysteine (pmole/mg)
200
150
100
50
0
***
**

A

Heart homogenates

↓

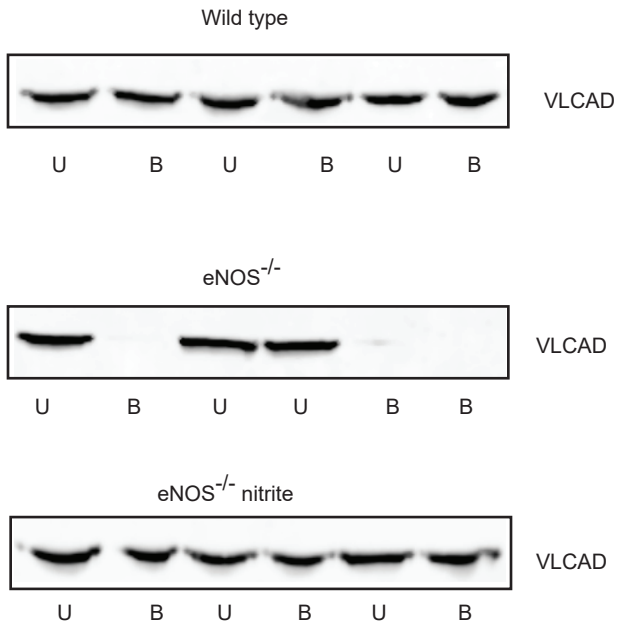
Oganomecury assisted capture

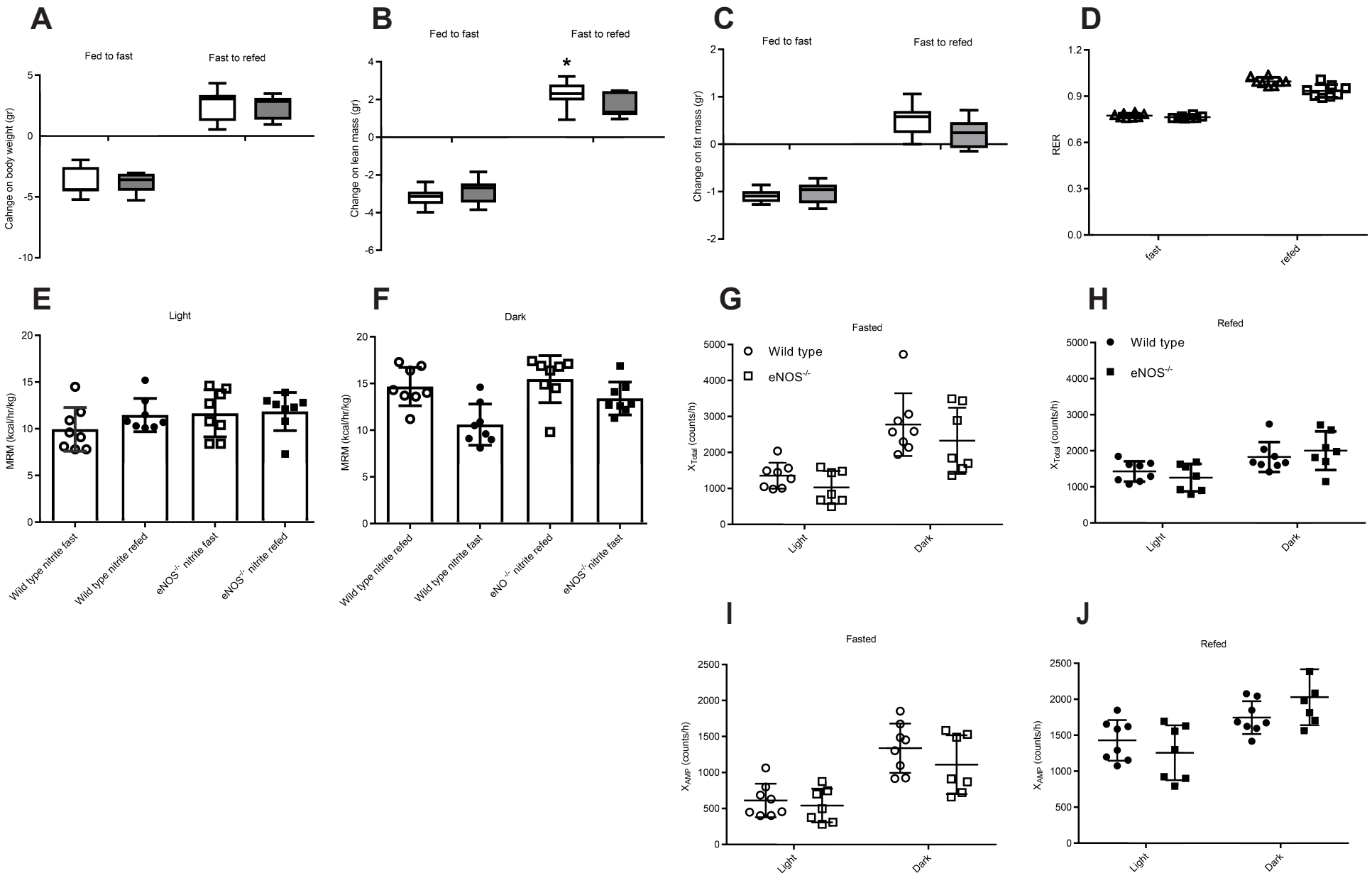
↓

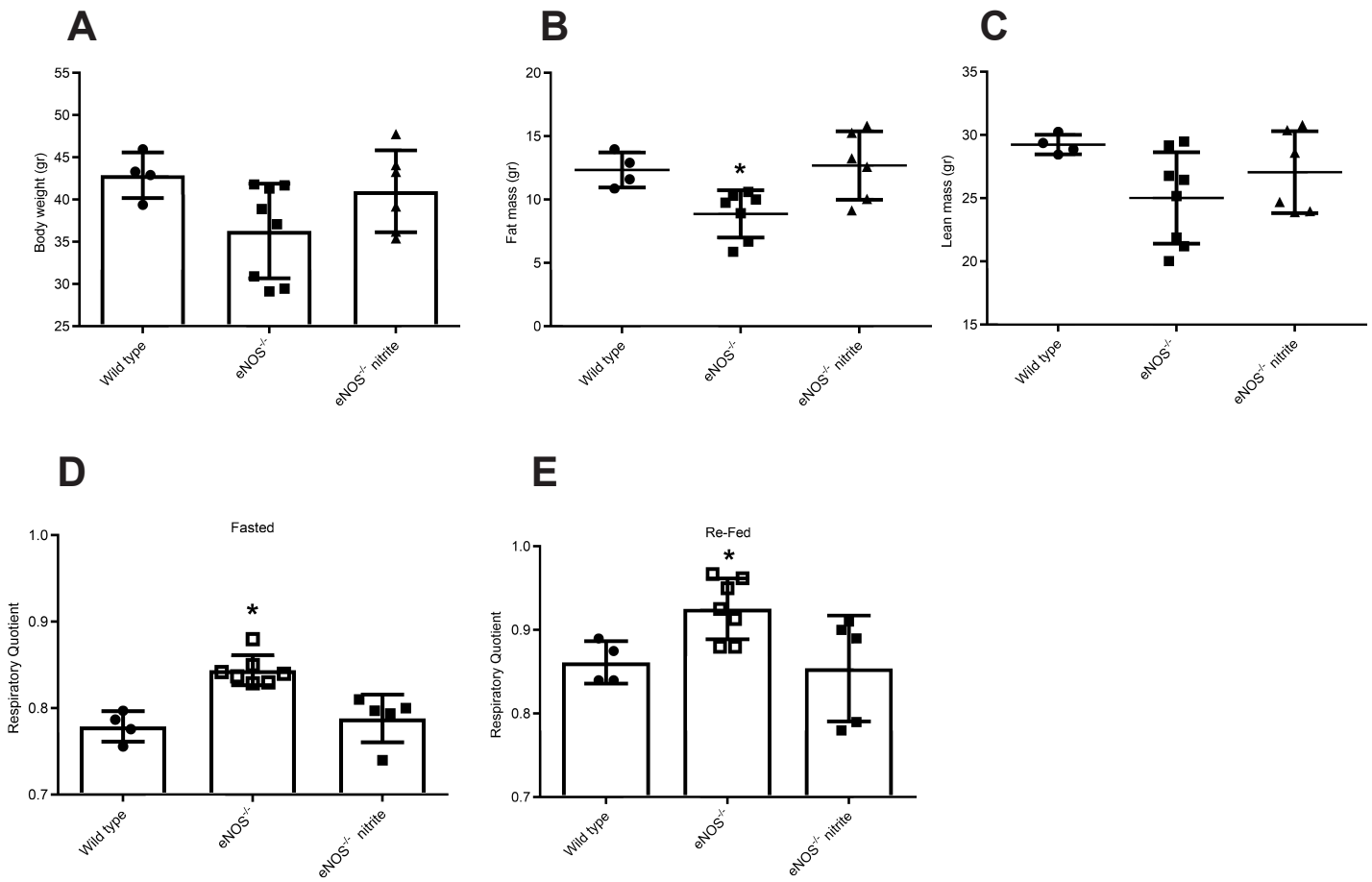
Western blot analysis

1. Unbound = unmodified
2. Bound = S-nitosation
3. Negative contol (cVLCAD$^{-/-}$ mice)

B




A
Fed to fast
Fast to refed
Cahnge on body weight (gr)
B
Fed to fast
Fast to refed
Change on lean mass (gr)
C
Fed to fast
Fast to refed
Change on fat mass (gr)
D
RER
fast
refed
E
Light
MRM (kcal/hr/kg)
Wild type nitrite fast
Wild type nitrite refed
eNOS-/- nitrite fast
eNOS-/- nitrite refed
F
Dark
MRM (kcal/hr/kg)
Wild type nitrite refed
Wild type nitrite fast
eNO-/- nitrite refed
eNOS-/- nitrite fast
G
Fasted
Wild type
eNOS-/-
XTotal (counts/h)
Light
Dark
H
Refed
Wild type
eNOS-/-
XTotal (counts/h)
Light
Dark
I
Fasted
XAMP (counts/h)
Light
Dark
J
Refed
XAMP (counts/h)
Light
Dark


A
Body weight (gr)
55
50
45
40
35
30
25
Wild type
eNOS-/-
eNOS-/- nitrite
B
Fat mass (gr)
20
15
10
5
0
*
Wild type
eNOS-/-
eNOS-/- nitrite
C
Lean mass (gr)
35
30
25
20
15
Wild type
eNOS-/-
eNOS-/- nitrite
D
Fasted
Respiratory Quotient
1.0
0.9
0.8
0.7
*
Wild type
eNOS-/-
eNOS-/- nitrite
E
Re-Fed
Respiratory Quotient
1.0
0.9
0.8
0.7
*
Wild type
eNOS-/-
eNOS-/- nitrite

Supplemental Table 1. Levels of liver acyl CoA species

Metabolite (pmol/mg)	**Wild type (N=3)**	**eNOS$^{-/-}$ (N=3)**
CoA-SH	87.1 ± 15.9	97.1 ± 14.1
Acetyl-CoA	40.3 ± 5.6	40.3 ± 7.0
Propionly-CoA	5.2 ± 1.1	3.5 ± 0.8
Butyryl-CoA	3.6 ± 0.3	2.8 ± 0.4
Succinyl-CoA	7.4 ± 1.2	4.8 ± 1.1^{A}
Acetoacetyl-CoA	0.3 ± 0.07	0.3 ± 0.04
3-HMG-CoA	0.2 ± 0.05	0.2 ± 0.03
3-HO-Butyrate-CoA	7.1 ± 2.9	7.1 ± 2.4
Acetoacetyl-CoA/ acetyl-CoA	0.008 ± 0.002	0.007 ± 0.0004
3-HGM-CoA/acetyl-CoA	0.005 ± 0.001	0.005 ± 0.0004

Values represent mean ± SD. Data were analyzed by1-way ANOVA, $^{A}p< 0.05$

Supplemental Table 2. Quantification of hepatic amino acids levels

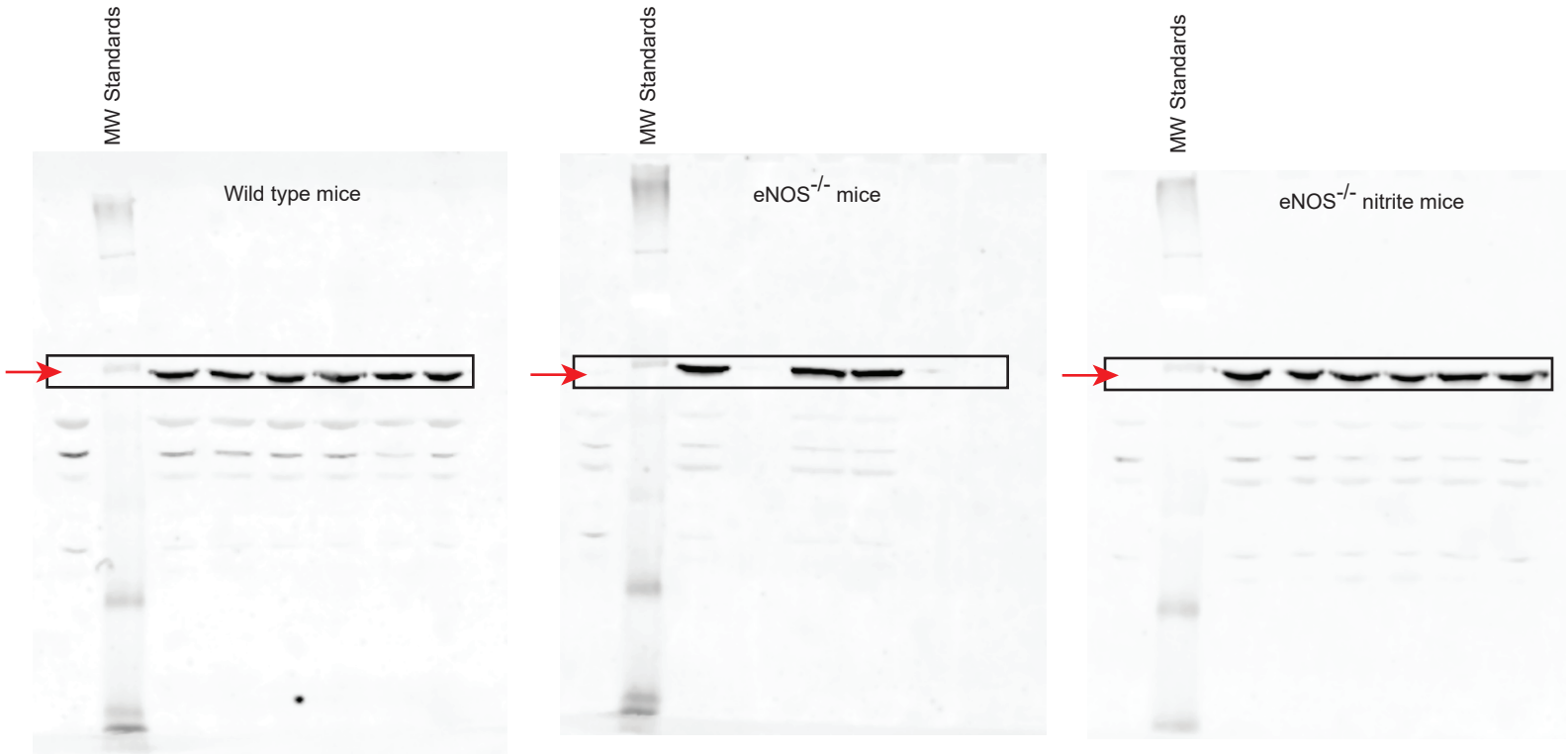
Amino acid (nmol/mg)	Wild type (N=4)	eNOS$^{-/-}$ (N=4)
ALA	1.82 ± 0.72	1.77 ± 0.24
ASN	0.14 ± 0.02	0.14 ± 0.03
ASP	0.38 ± 0.08	0.43 ± 0.24
CIT	0.04 ± 0.01	0.03 ± 0.01
GLN	1.62 ± 0.12	1.44 ± 0.32
GLU	0.76 ± 0.14	1.05 ± 0.27
GLY	1.36 ± 0.26	1.32 ± 0.25
ORN	0.27 ± 0.11	0.25 ± 0.05
SER	0.19 ± 0.03	0.18 ± 0.06
TAU	2.84 ± 0.22	2.85 ± 0.28
THR	0.17 ± 0.03	0.18 ± 0.04
TYR	0.04 ± 0.01	0.04 ± 0.01
VAL	0.15 ± 0.04	0.18 ± 0.06
HIS	1.45 ± 0.04	1.33 ± 0.12
LYS	0.47 ± 0.13	0.41 ± 0.17
PHE	0.03 ± 0.01	0.04 ±0.01
LEU	0.16 ± 0.03	0.18 ±0.03
TRY	0.02 ±0.01	0.03 ± 0.01
ILE	0.07 ±0.02	0.08 ±0.03

Values represent mean ± SD.

Supplemental Table 3. Quantification of serum acylcarnitines

Acylcarnitine (nmol/L)	Wild type (N=4)	eNOS$^{-/-}$ (N=4)	eNOS$^{-/-}$+ nitrite (N=4)
C2	3009± 249	3624 ± 805	2602 ± 680
C3:1	3.3 ± 2.5	2.1 ± 2.0	1.5 ± 1.0
C3	31 ± 7	46 ± 19	33 ± 19
C4	22 ± 3	34 ± 9	33 ± 9
C5:1	1.2 ± 0.01	1.2 ± 0.1	0.5 ± 0.6
C5	9.9 ± 2.3	13 ± 4	8.0 ± 2.9
C4-OH	40 ± 7	57 ± 8	52 ± 6
C6	13 ± 1	17 ± 6	6.8 ± 2.6
C5-OH	7.5 ± 1.1	9.0 ± 1.5	5.8 ± 1.2
C8:1	1.5 ± 0.6	1.5 ± 0.6	1.2 ± 0.5
C8	2.4 ± 0.01	3.3 ± 1.1	2.5 ± 0.6
C3-DC	6.6 ± 0.7	8.4 ± 2.0	6.2 ± 1.2
C10:2	2.1 ± 1.1	1.8 ± 0.7	1.5 ± 0.6
C10:1	3.0 ± 0.7	4.2 ± 0.7	3.0 ± 0.8
C10	2.7 ± 0.6	4.2 ± 1.5	4.0 ± 1.4
C4-DC	8.1 ± 4.3	8.7 ± 4.0	4.5 ± 1.0
C5-DC	4.2 ± 1.5	4.8 ± 1.0	3.8 ± 1.0
C12:1	2.7 ± 0.6	4.2 ± 0.7	2.5 ± 0.6
C12	6.0 ± 1.4	8.7 ± 2.0	5.8 ± 1.7
C6-DC	7.8 ± 2.3	13 ± 5	9.5 ± 2.6
C14:2	6.3 ± 1.8	8.7 ± 2.0	5.5 ± 1.9
C14:1	16 ± 5	28 ± 4	16 ± 4
C14	22 ± 4	33 ± 6	20 ± 4
C8-DC	3.0 ± 0.7	4.2 ± 2.1	2.8 ± 1.0
3-OH-C12:1	1.7 ± 0.1	2.4 ± 0.1	2.2 ±1.0
3-OH-C12	3.1 ± 1.4	6,6 ± 3.7	11 ± 6
3-OH-C14:1	3.8 ± 1.1	8.1 ± 1.1	5.0 ± 1.4
3-OH-C14	2.7 ± 0.8	4.8 ± 1.7	4.0 ± 1.0
C16:2	4.0 ± 1.4	9.6 ± 1.0	5.8 ± 1.7
C16:1	10 ± 3	26 ± 3	19 ± 3
C16	117 ± 17	195 ± 50	111 ± 21
3-OH-C16	3.8 ± 1.1	6.9 ± 1.1	5.8 ± 1.2
C18:2	17 ± 4	35 ± 4	20 ± 5
C18:1	43 ± 11	99 ± 13	68 ± 13
C18	12 ± 3	25± 5	14 ± 4
OH-C18:2	2.3 ± 1.0	4.2 ± 0.7	3.0 ± 0.8
OH-C18:1	5.8 ± 2.5	12 ± 2	7.8 ±1.7
C16-DC	0.8 ± 0.1	1.2 ± 0.1	1.0 ± 0.8
C18:1-DC	0.8 ± 0.1	1.2 ± 0.1	1 ± 0.1

Values represent mean ± SD.



1. Three biological eplicates were tested per group. Upon the collection of bound and unbound fractions the samples were concentrated and loaded on the gels.

2. The specificity of the anti-VLCAD antibody was tested in cardiac specific VLCAD$^{-/-}$ mice. These lanes are indicated by an arrow.
The absence of immunoreactivity in these preparations documented the specificity of the antibody.