Supplemental Information

# Partial loss of endothelial nitric oxide leads to increased cerebrovascular beta amyloid

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Supplemental Table, Figures and Figure Legends

**Supplemental Table 1.** Peripheral parameters of 18 month old wild type and $eNOS^{+/-}$ mice. Data is presented as mean ±SD (*P<0.05).

**Supplemental Figure 1.** Expression of APP, BACE1, and ADAM10 were unaltered in brain tissue. (a) Brain tissue from 18 month old $eNOS^{+/-}$ and wild type animals was Western blotted using anti-APP, anti-BACE1, anti-ADAM10, and anti-Actin (loading control) antibodies. Representative image is shown. Densitometric analysis was performed for (b) APP, (c) BACE1, and (d) ADAM10 (n=6-7 animals). BACE enzymatic activity was measured from 18 month old $eNOS^{+/-}$ and wild type (e) brain tissue via a commercially available BACE activity assay kit (n=3-4; *P<0.05). All data are presented as mean ±SD with individual data points shown.

**Supplemental Figure 2.** Expression of eNOS was significantly decreased in microvascular tissue of $eNOS^{+/-}$ mice. (a) Microvascular tissue from 18 month old $eNOS^{+/-}$ and wild type animals was analyzed by Western blot analyses for NOS isoforms. Representative image is shown. Densitometric analysis was performed for (b) eNOS (n=3 animals; *P<0.05) and (c) iNOS (n=8 animals). All data are presented as mean ±SD with individual data points shown.

**Supplemental Figure 3.** Expression of COX-2 was significantly higher in 18 month old $eNOS^{+/-}$ microvascular tissue. (a) Brain tissue from 18 month old $eNOS^{+/-}$ and wild type animals was Western blotted using anti-COX-1, anti-COX-2, anti-$PGI_2S$, anti-$TXA_2S$, and anti-Actin (loading control) antibodies. Representative image is shown. Densitometric analysis was performed for (b) COX-1 (n=7 animals), (c) COX-2 (n=5-6 animals; *P<0.05), (d) $PGI_2S$ (n=6 animals), and (e) $TXA_2S$ (n=12 animals). All data are presented as mean ±SD with individual data points shown.

**Supplemental Figure 4.** Expression of eNOS was significantly decreased while other NOS isoforms were unchanged in brain tissue of $eNOS^{+/-}$ mice. (a) Brain tissue from 18 month old $eNOS^{+/-}$ and wild type animals was analyzed by Western blot analyses for NOS isoforms. Representative image is shown. Densitometric analysis was performed for (b) eNOS, (c) iNOS,

and (d) nNOS (n=7 animals; ***P<0.0001). All data are presented as mean ±SD with individual data points shown.

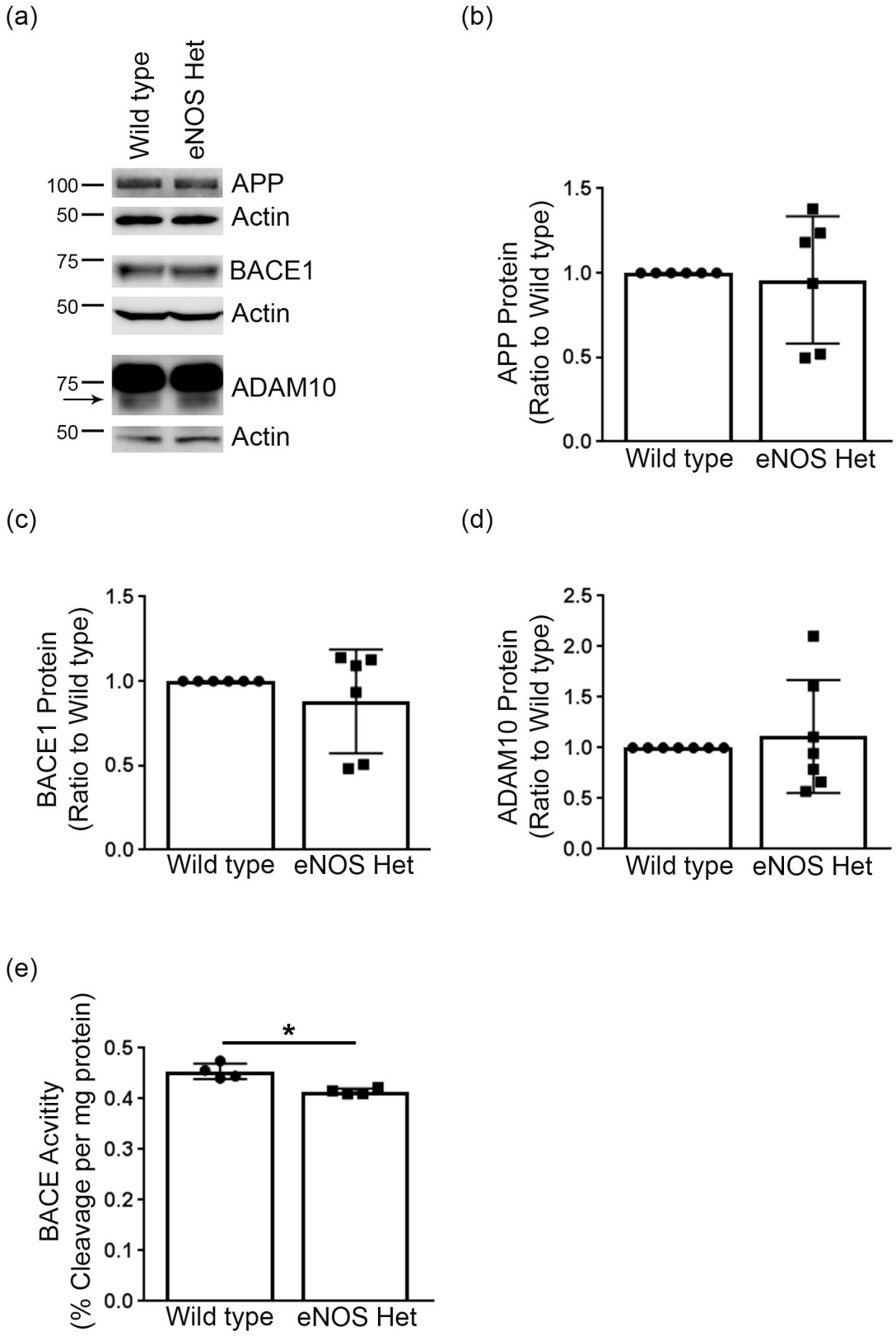
**Supplemental Figure 5.** IDE was significantly increased in 18 month old eNOS$^{+/-}$ brain tissue. (a) Brain tissue from 18 month old eNOS$^{+/-}$ and wild type animals was Western blotted using anti-ECE1, anti-IDE, anti-neprilysin, and anti-Actin (loading control) antibodies. Representative image is shown. Densitometric analysis was performed for (b) ECE1 (n=7 animals), (c) IDE (n=9 animals; *P<0.05), and (d) neprilysin (n=5 animals). All data are presented as mean ±SD with individual data points shown.

**Supplemental Figure 6.** Expression of Aβ receptors, LRP1 and RAGE, were unaltered in brain tissue of eNOS$^{+/-}$ mice. (a) Brain tissue from 18 month old eNOS$^{+/-}$ and wild type animals was analyzed by Western blot analyses for Aβ receptors: LRP1 and RAGE. Representative image is shown. Densitometric analysis was performed for (b) LRP1 and (c) RAGE (n=7 animals). All data are presented as mean ±SD with individual data points shown.

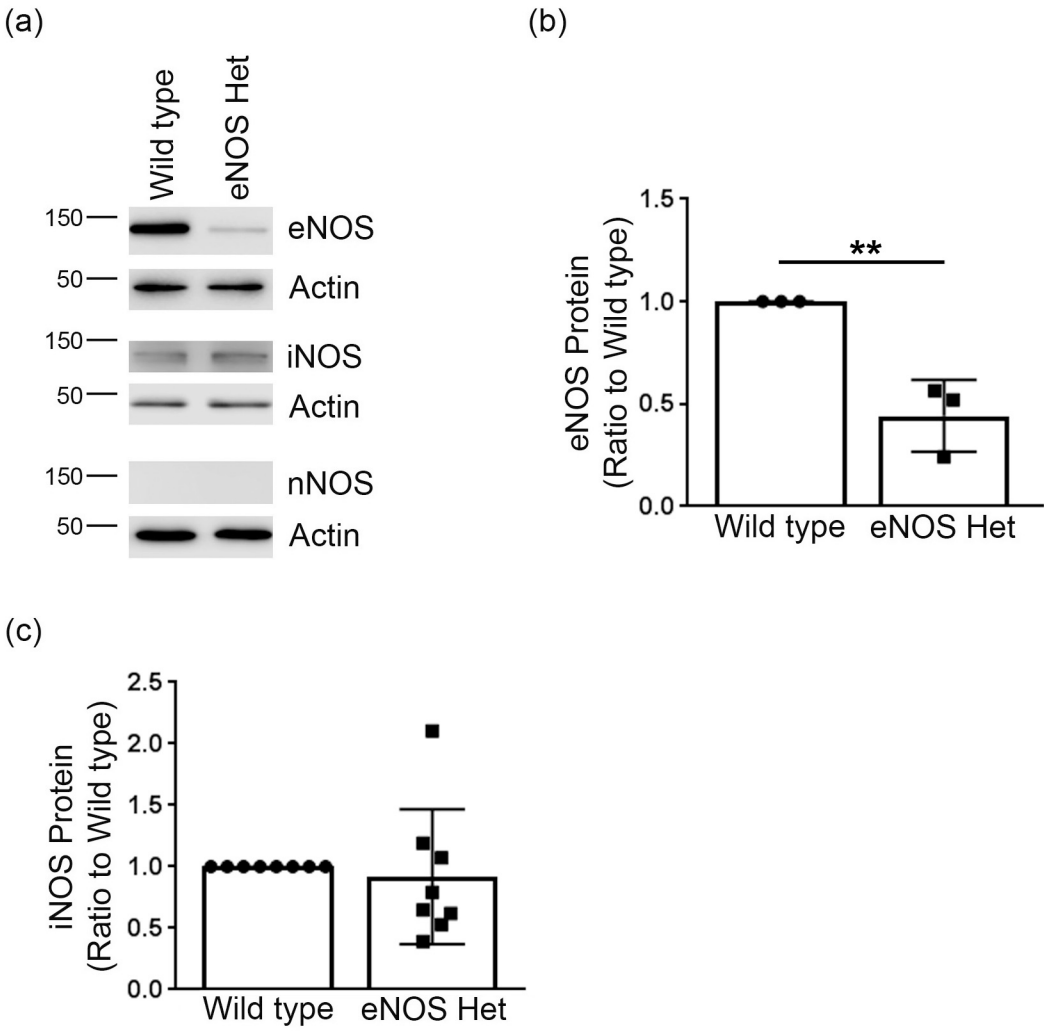
Supplemental Table 1. Characteristics of 18 month old wild type and $eNOS^{+/-}$ mice.

| Parameter | Wild type | $eNOS^{+/-}$ | |
|---|---|---|---|
| Body weight (g) | 35.29±4.62 | 38.35±6.27 | N=18 |
| Cholesterol, Total (mg/dL) | 70.40±8.17 | 67.60±5.32 | N=5 |
| HDL (mg/dL) | 51.80±8.70 | 50.20±4.87 | N=5 |
| Triglycerides (mg/dL) | 78.40±14.29 | 99.75±11.87* | N=4-5 |
| Glucose (mg/dL) | 189.24±39.60 | 199.29±31.63 | N=17 |
| sAPPα (pg/mL) | 523.88±65.53 | 575.07±50.97 | N=5 |
| Aβ40 (pg/mL) | 220.70±68.79 | 216.91±23.85 | N=7 |
| Aβ42 (pg/mL) | 8.72±3.72 | 14.06±5.54 | N=4-6 |

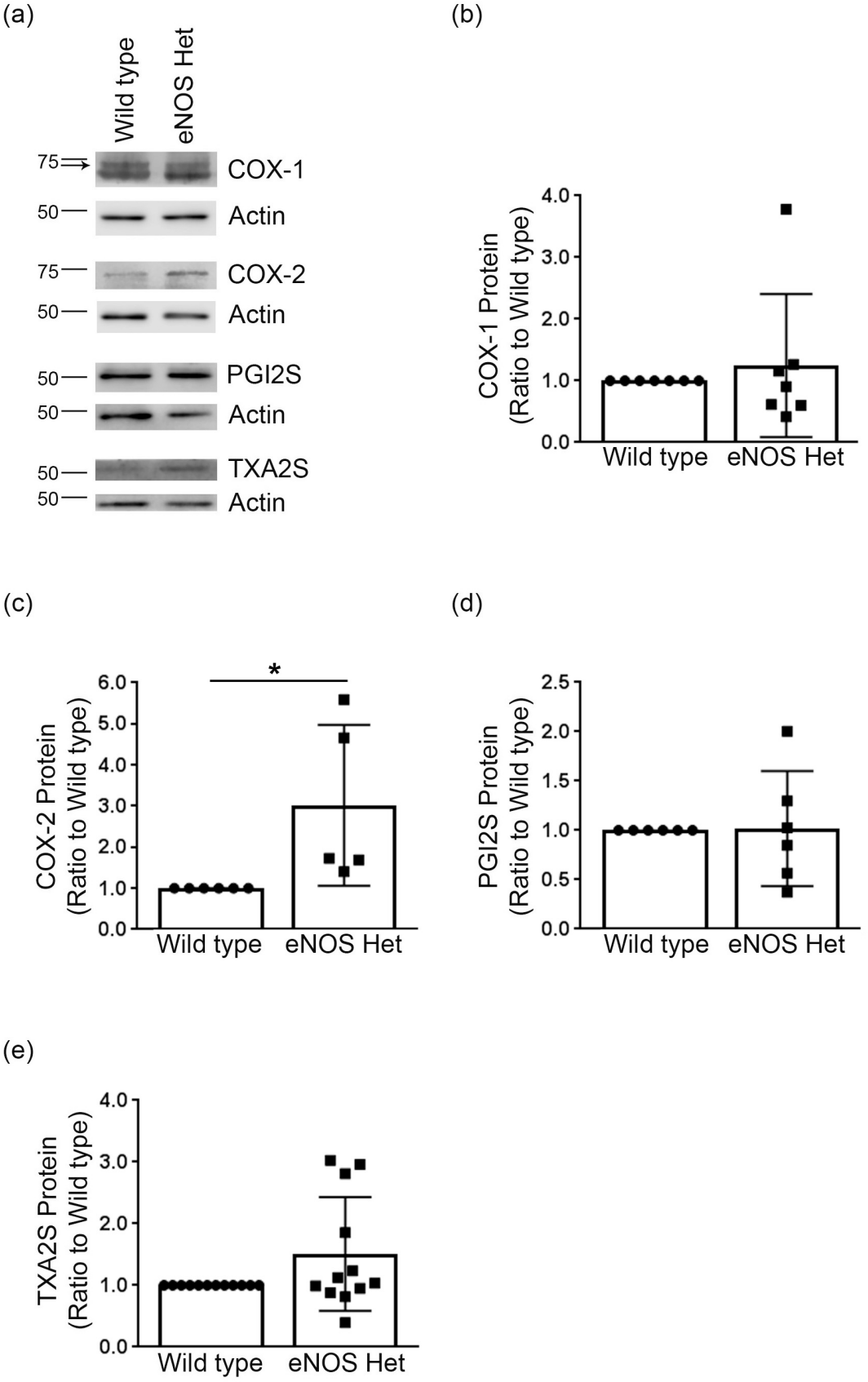
Data presented as mean ± SD (*$p<0.05$).



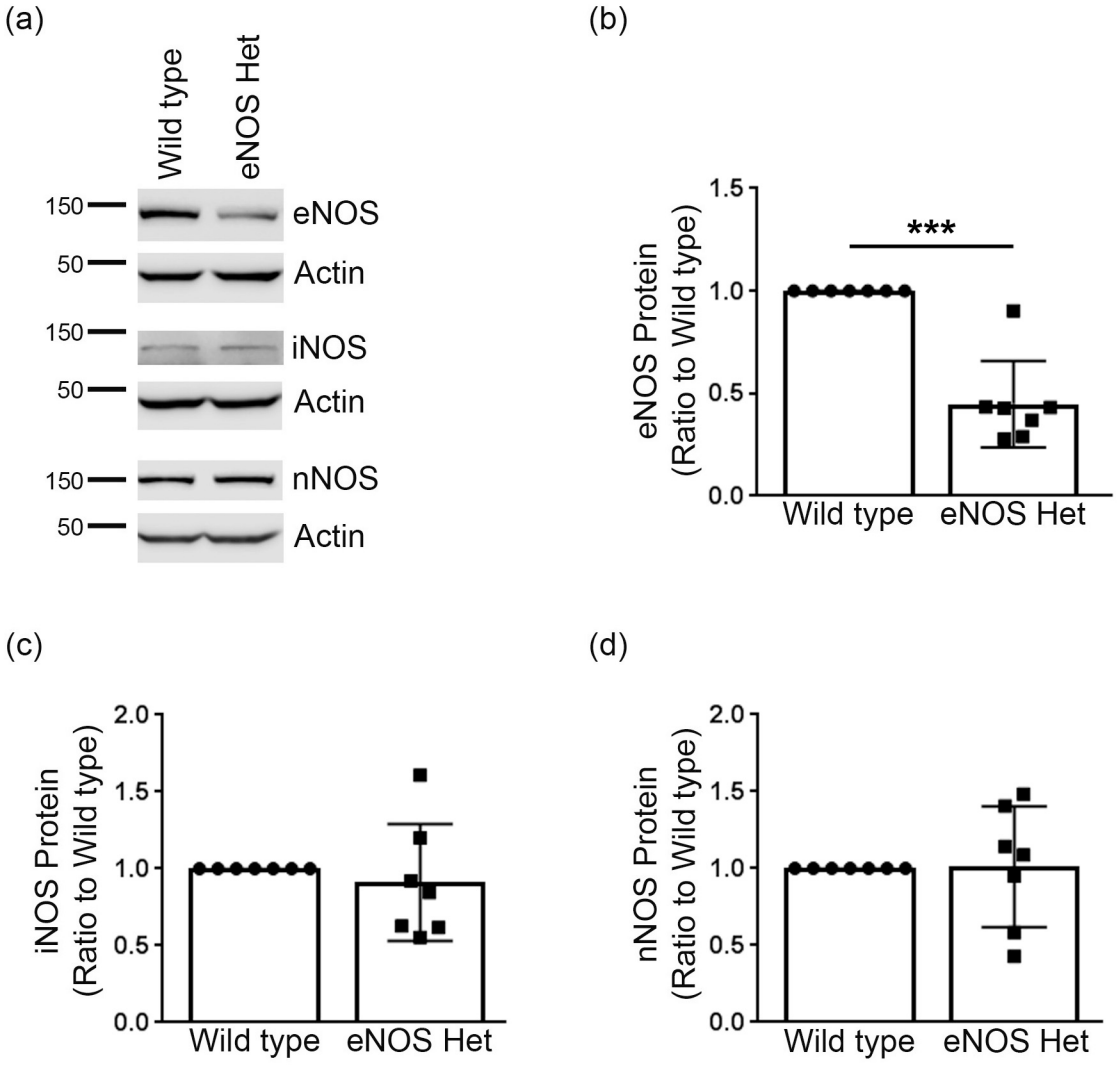

Supplemental Figure 1



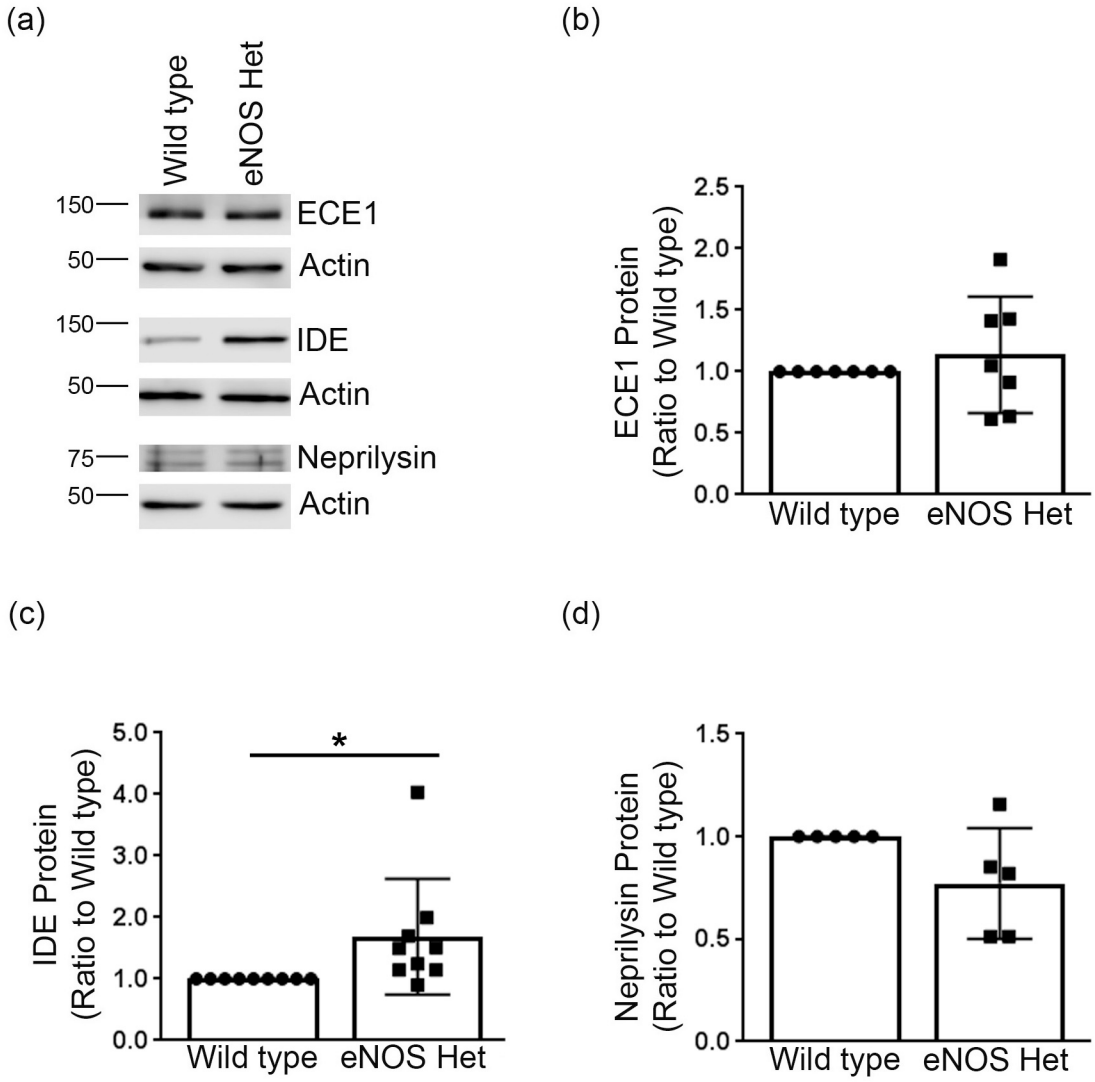

Supplemental Figure 2


(a)
Wild type
eNOS Het
75
COX-1
50
Actin
75
COX-2
50
Actin
50
PGI2S
50
Actin
50
TXA2S
50
Actin
(b)
COX-1 Protein
(Ratio to Wild type)
4.0
3.0
2.0
1.0
0.0
Wild type
eNOS Het
(c)
*
COX-2 Protein
(Ratio to Wild type)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Wild type
eNOS Het
(d)
PGI2S Protein
(Ratio to Wild type)
2.5
2.0
1.5
1.0
0.5
0.0
Wild type
eNOS Het
(e)
TXA2S Protein
(Ratio to Wild type)
4.0
3.0
2.0
1.0
0.0
Wild type
eNOS Het

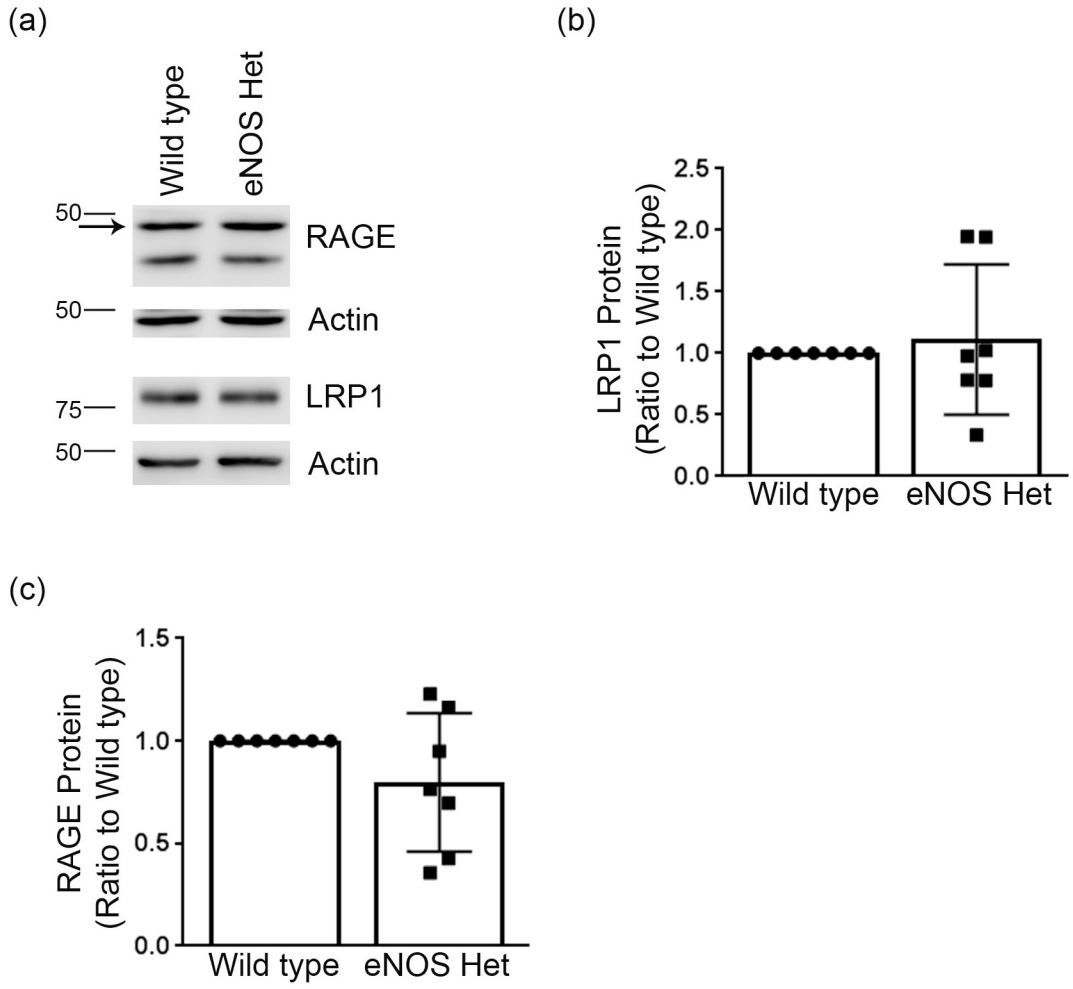
Supplemental Figure 3




Supplemental Figure 4




Supplemental Figure 5




Supplemental Figure 6