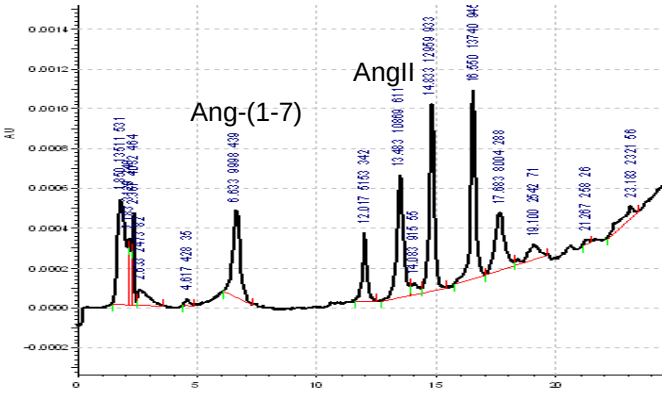
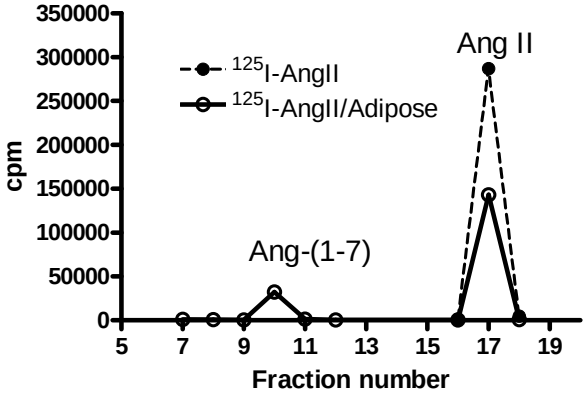
Table I. Primer sequences

Gene	Primer sequence	Product size
ACE2	5'TGTGTCAGAAATGTGCGCTTC3' 5'CAAGGCGTATCTGTCACAGTC3'	175
AGT	5'GAAACCTCTCATCGTTCCTTG3' 5'TCTCTTTACCCCTGCCCTCT3'	193
PPARγ	5'GATGGAAGACCACTCGCATT3' 5'AACCATTGGGTCAGCTCTTG3'	115
18SRNA	5'AGTCGGCATCGTTTATGGTC3' 5'CGAAAGCATTTGCCAAGAAT3'	151

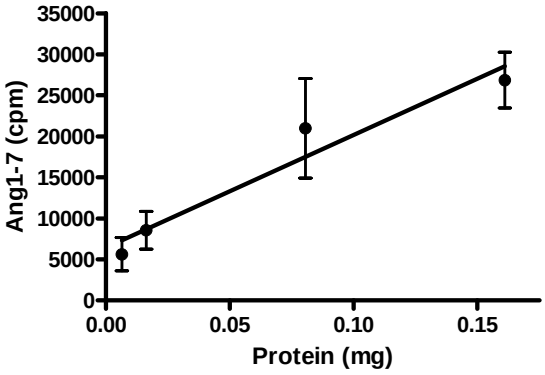
AGT = angiotensinogen

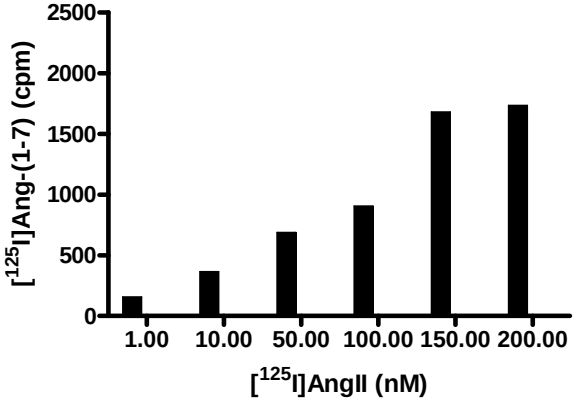
A



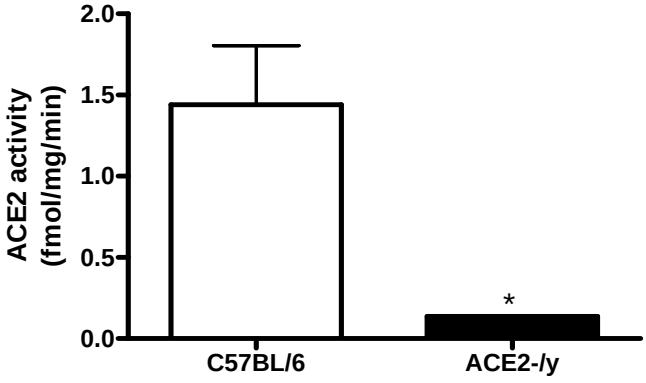


B



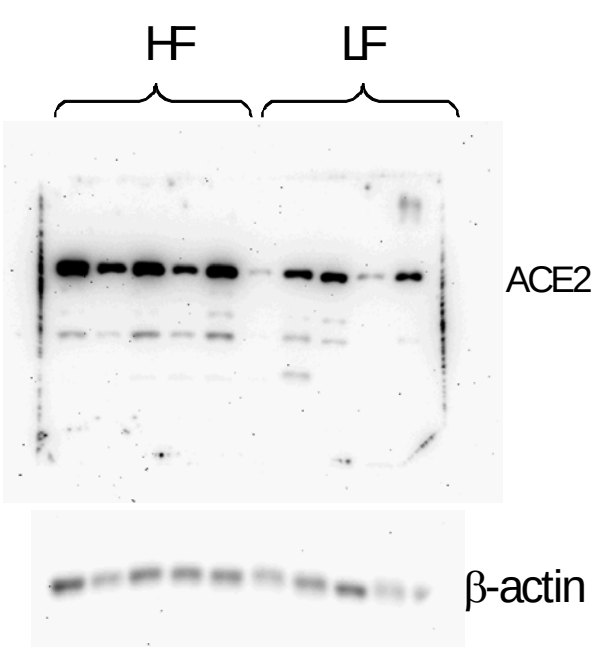


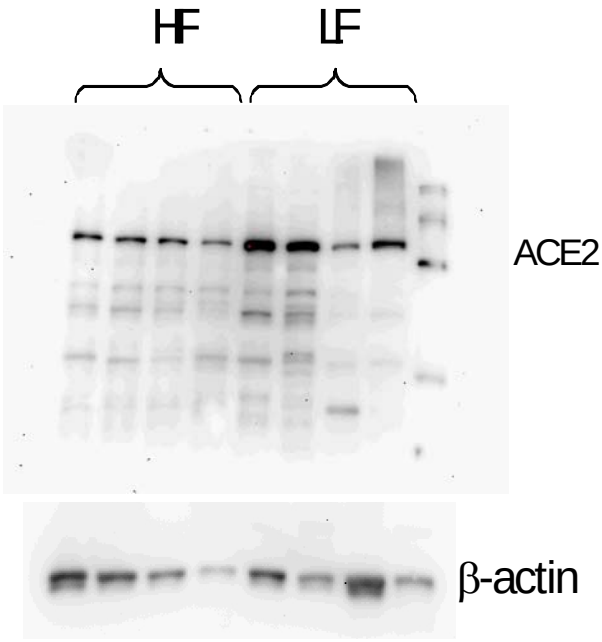
C

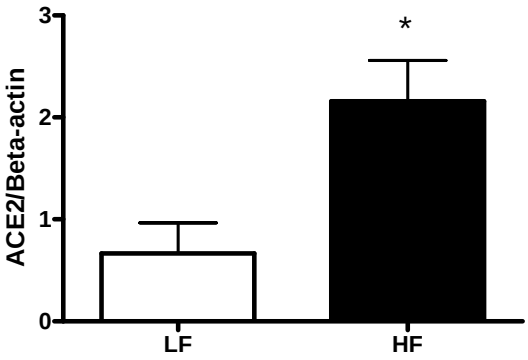


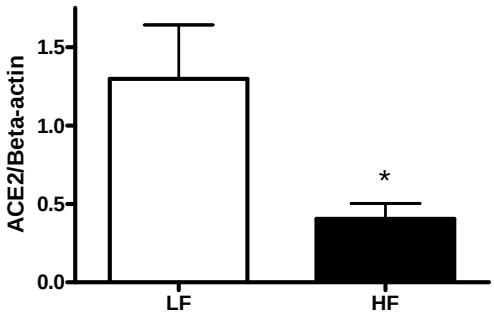
Supplemental Figure 1.

Supplemental Figure 1. ACE2 activity measurement. **A**, Left, HPLC chromatogram for resolution of angiotensin 1-7 (Ang-(1-7); retention time = 6.6 min) and angiotensin II (AngII; retention time = 13.6 min). Other angiotensin peptides resolved using this protocol include angiotensin III, angiotensin IV, angiotensin I and angiotensin4-8. Right, Effect of protease inhibitors on Ang-(1-7) generation. When [^{125}I]AngII is injected onto the HPLC in the absence of adipose membrane ([^{125}I]AngII), only one peak of radioactivity is demonstrated in HPLC fractions. In the presence of adipose membrane ([^{125}I]AngII/adipose), radioactivity is detected in the Ang-(1-7) fraction, but not in other HPLC fractions. Radioactivity appears in HPLC fractions with a 3 minute lag time from the retention time of individual peptides. **B**, Left, ACE2 activity increases as adipose membrane protein increases. A membrane protein concentration of 0.05 mg was chosen for studies. Right, Dependence of [^{125}I]Ang-(1-7) product formation from varying concentrations of [^{125}I]AngII substrate. Measurements were performed at a saturating concentration of [^{125}I]AngII substrate (200 nM). Moreover, under these experimental conditions, less than 5% of [^{125}I]AngII substrate was hydrolyzed to [^{125}I]Ang-(1-7), to assure that substrate concentrations were not limiting. **C**, ACE2 activity is markedly decreased in EF membranes from ACE2-/y mice compared to control (C57BL/6).


HF
LF
ACE2
β-actin


HF
LF
ACE2
β-actin


ACE2/Beta-actin
3
2
1
0
*
LF
HF


ACE2/Beta-actin
1.5
1.0
0.5
0.0
*
LF
HF

Supplemental Figure 2. ACE2 protein in EF from 1 week and 4 month LF and HF-fed mice. Western blotting for ACE2 was as described in methods. Expression of ACE2 increased in 1 week HF compared to LF mice, while at 4 months ACE2 protein was reduced in adipose tissue from HF-fed mice. Top, Representative western blots for ACE2 and β-actin in EF membranes from LF and HF-fed mice. Bottom, Densitometry for ACE2 expression normalized to β-actin. Data are mean ± SEM from n = 10 mice/group. *, denotes significantly different from LF, $P < 0.05$.