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**Article:**

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<https://doi.org/10.1161/CIRCRESAHA.116.309678>

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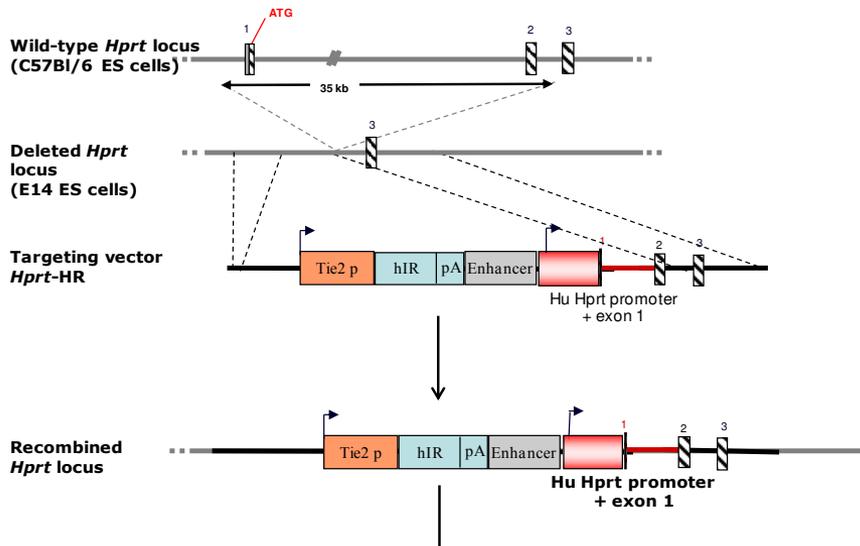
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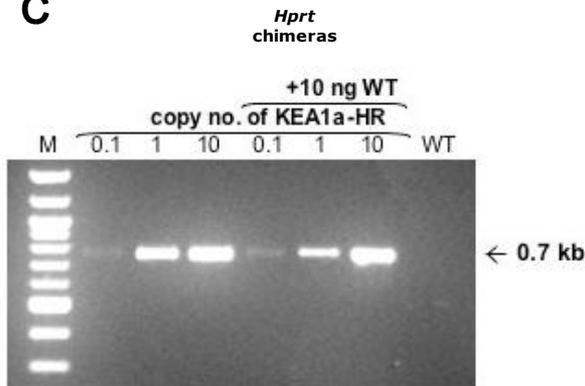
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# Supplemental Figure I

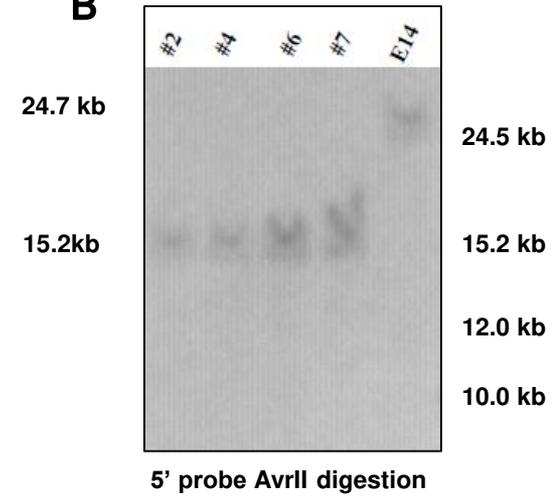
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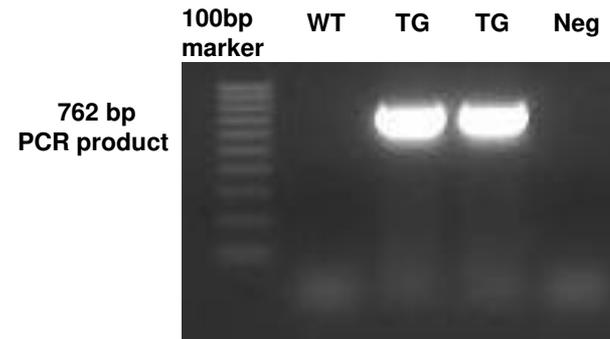
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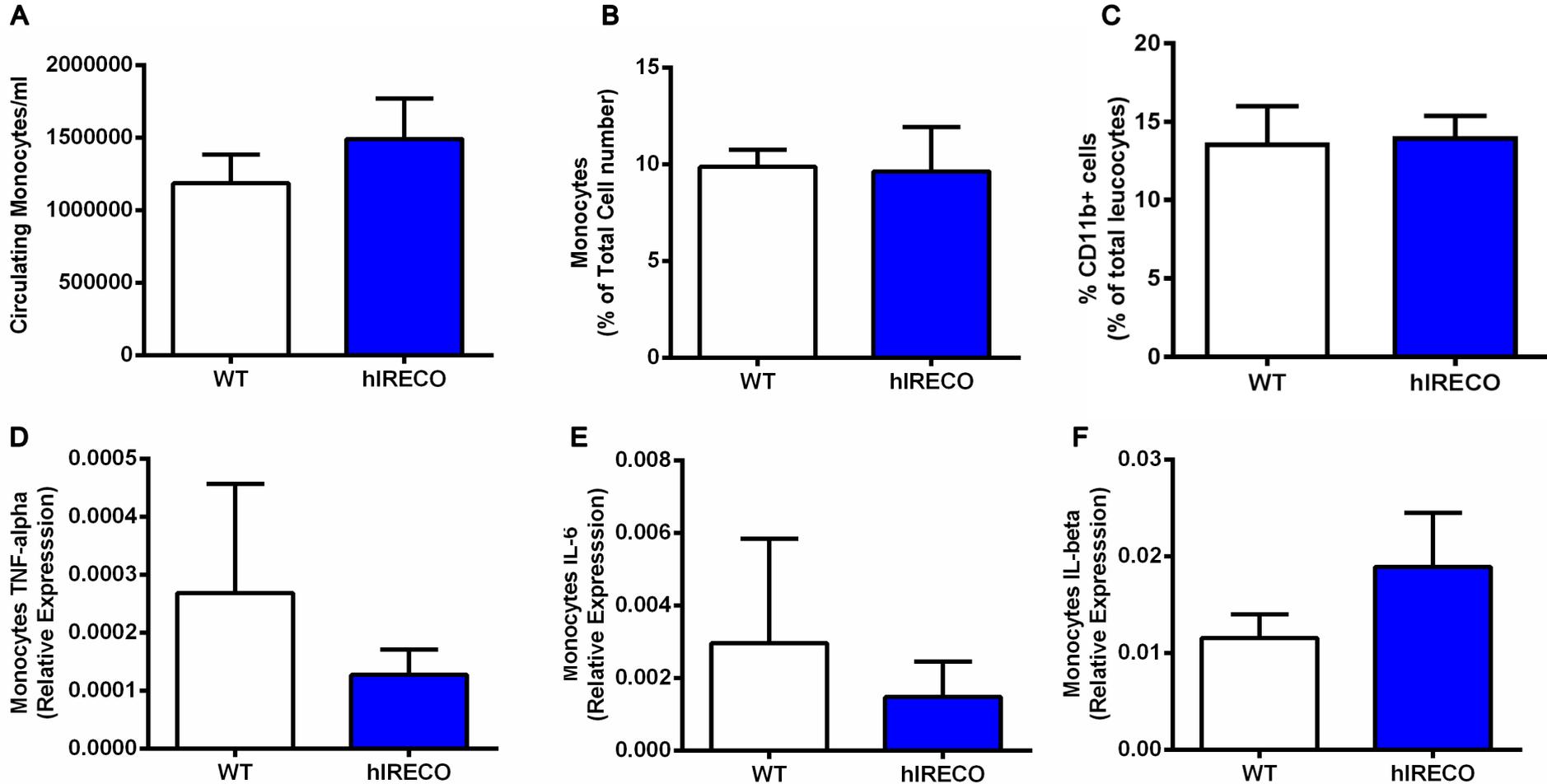


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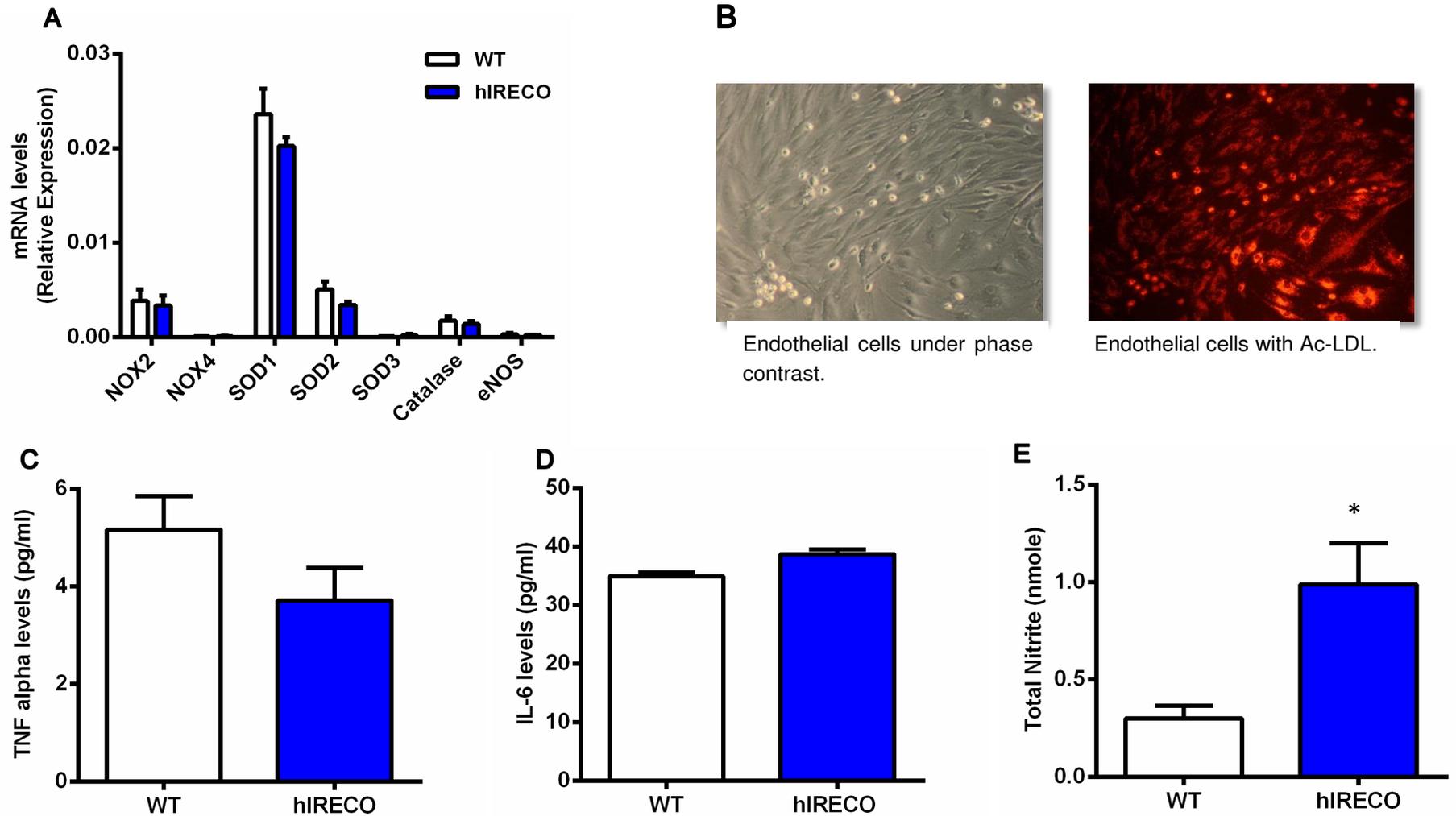
**Supplemental Figure I. A)** Construction of targeting vector to generate mice with endothelium-specific transgenic expression of human insulin receptor. **B)** Southern blot validated the correct heterozygous status of 4 of 7 tested F1 females, by detecting the 15.2 kb sized AvrII fragment of the C57BL/6 *Hprt* wild type allele and the 9.8 kb sized AvrII fragment of the reconstituted *Hprt* allele. **C)** Genotyping protocol validated the multiple copies of transgene. **D)** PCR product using isolated DNA, showing no human insulin receptor gene expression in wild type (WT) and in hIRECO (TG) ear notches. Neg denotes negative control, using water as template.

## Supplemental Figure II



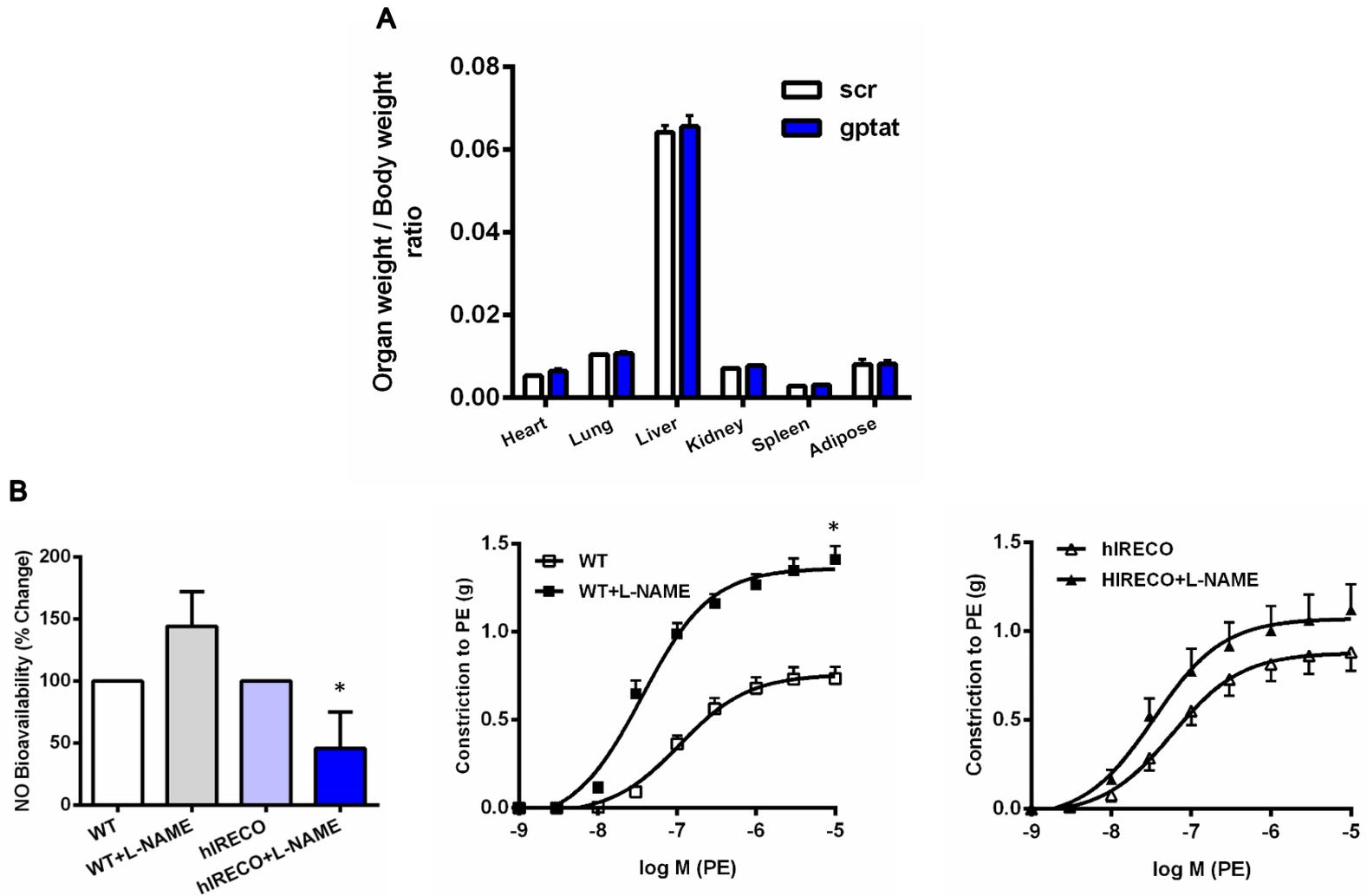
**Supplemental Figure II.** **A)** No difference in monocyte count in mice with endothelial cell specific over-expression of the human insulin receptor (hIRECO). **B)** No difference in monocyte as percent of circulating cells in hIRECO. **C)** No difference in CD11b expression in monocytes from hIRECO. **D)** No difference in TNF-alpha mRNA expression in monocytes from hIRECO. **E)** No difference in IL-16 mRNA expression in monocytes from hIRECO. **F)** No difference in IL-1Beta mRNA expression in monocytes from hIRECO (Data presented as mean  $\pm$  SEM. All experiments n=5 mice WT denotes wild type).

### Supplemental Figure III



**Supplemental Figure III. A)** No difference in mRNA expression of Nox2 NADPH oxidase (Nox2), Nox4 NADPH oxidase (Nox4) in superoxide dismutase 1,2 and 3 (SOD), catalase and endothelial NO synthase in pulmonary endothelial cells from mice with endothelial cell specific over-expression of the human insulin receptor (hIRECO). **B)** Isolated pulmonary endothelial cells under phase contrast microscopy and upon staining with Dil-conjugated Acetylated LDL (Ac-LDL) **C)** No difference in circulating plasma TNF- $\alpha$  in hIRECO. **D)** No difference in circulating plasma IL-6 in hIRECO **E)** Total nitrite levels are significantly higher in the hIRECO plasma compared to WT (all experiments n=5 mice WT denotes wild type).

## Supplemental Figure IV



**Supplemental Figure IV. A)** Chronic pharmacological inhibition of Nox2 NADPH oxidase with gp91ds-tat to hIRECO mice via osmotic mini-pump for 28 days had no effect on organ mass. **B)** Basal NO production in response to isometric tension assessed by measuring the constrictor response to the NO synthase inhibitor, L-NMMA was reduced in hIRECO mice (left). PE constrictor response in the WT with and without L-NMMA (centre). PE constrictor response in hIRECO mice aorta with and without L-NMMA (right). % Change means the change from maximal constriction to PE after L-NMMA (Data presented as mean  $\pm$  SEM. All experiments n=5 mice WT denotes wild type).

**Supplemental Table I. Primer sequences used for quantitative PCR for mRNA levels**

<b>Gene</b>	<b>Forward primer</b>	<b>Reverse primer</b>
<i>Beta-Actin</i>	CGTGAAAAGATGACCCAGATCA	TGGTACGACCAGAGGCATACAG
<i>NOX2</i>	GGTCCAGTGCCTGTTGCT	GCGGTGTGCAGTGCTATCAT
<i>NOX4</i>	GGAGACTGGACAGAACGATTCC	TGTATAACTTAGGGTAATTTCTAGAGTGAATGA
<i>SOD1</i>	GGACCTCATTTTAATCCTCACTCTAAG	GGTCTCCAACATGCCTCTCTTC
<i>SOD2</i>	CACACATTAACGCGCAGATCA	GGTGGCGTTGAGATTGTTCA
<i>SOD3</i>	GGGATGGATCTAGAGCATTAAAGGA	ACACCTTAGTTAACCCAGAAATCTTTTC
<i>eNOS</i>	CTGGAGCACCCACGCT	AGCGGTGAGGGTCACACAG
<i>Catalase</i>	GCTGAGAAGCCTAAGAACGCAAT	CCCTTCGCAGCCATGTG
<i>Human Insulin Receptor</i>	GTCATCAACGGGCAGTTTG	GGTGCAGCCGTGTGACTTAC
<i>Mouse Insulin Receptor</i>	CTT GAT GTG CAC CCC ATG TCT	TCG GAT GTT GAT GAT CAG GCT
<i>Mouse ve-Cadherin</i>	TCAACGCATCTGTGCCAGAGAT	CACGATTTGGTACAAGACAGTG
<i>Mouse TNF-alpha</i>	Taqman probes: mm00443258_m1	
<i>Mouse IL-6</i>	Taqman probes: mm00446190_m1	
<i>Mouse IL-1beta</i>	Taqman probes: mm00434228_m1	