Supplemental Material and Methods

Blunting of cardioprotective actions of estrogen in female rodent heart linked to altered expression of cardiac tissue chymase and ACE2

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Real-time PCR
Real-time PCR was used to quantify mRNA expression for specific genes in LV tissue that are listed below. Total RNA was extracted from frozen, pulverized LV tissue from each group using TRIzol reagent, and processed according to the manufacturer’s recommendations. The quality and quantity of RNA samples were determined by spectrometry and agarose gel electrophoresis. Complementary first-strand DNA was synthesized from oligo (dT)-primed total RNA, using the Omniscript RT kit (Qiagen Inc, Valencia, CA). Relative quantification of mRNA levels by real-time PCR was performed using a SYBR Green PCR kit (Qiagen Inc). Amplification and detection were performed with the ABI7500 Sequence Detection System (Applied Biosystems). Only one peak from the dissociation curve was found from each pair of oligonucleotide primers tested. Real-time PCR was carried out in duplicate; a no-template control was included in each run to check for contamination. It was also confirmed that no amplification occurred when samples were not subjected to reverse transcription. Sequence-specific oligonucleotide primers were designed according to published GenBank sequences (www-ncbi-nlm-nih-gov.go.libproxy.wakehealth.edu/Genbank) and confirmed with OligoAnalyzer 3.0. The relative target mRNA levels in each sample were normalized to S16 ribosomal RNA. Expression levels are reported relative to the geometric mean of the control group.

Table 1. Primer Sequences for Real-time PCR
| Gene name  | Sequence                                      | Product Size | Accession Number |
|-----------|-----------------------------------------------|--------------|------------------|
| ACE       | TTGACGTGAGCAACTTCCAG TGGTACGATCAGGCTCCAGTG   | 194          | AF539425         |
| Chymase   | TCTGGAGGACCTCCTCTCTGTT TGGCATTGGATGTCATAGG   | 66           | NM_013092        |
| Angiotensinogen | AATTCGGGGACCTACAACC CTCAGCACCCTAAAGGGTAG | 70           | XM_008772597     |
| AT1aR     | TCCTGCCACATTCCCTGAGTT CGAATCCACTGGCTGTTG    | 116          | M74054           |
| ACE2      | CGCTGTACCAGACAGAAAAGCCAACAGACAGAAGCCAGAGGA | 139          | NM_001012006     |
| MAS       | ACTGTCGGACGCTGCTCAATC GCATTCGGATGTACGTAGG   | 263          | XM_006227868     |
| ANF       | AGGGCTTCTTCTCTCTTCTGG CCAGGTGGTCTAGCAGGTTG | 117          | NM_012612        |
| BNP       | GCCAGTCTCCAGAACAATCC CCTTCCTGCTAGCAGGTTG   | 97           | NM_031545        |
| NOX2      | CGAAAAACTTCTTGGGTCTACGC AGGAGATTCCAGACACTGG | 101          | AF298656         |
| SERCA2    | ACCTCATTCTCTCCACGCTG GGGGTTGCTTTCTGATGTC   | 175          | NR_027839        |
| Phospholamban | TGACGATCAGAAGCCAAGG CCGAGCGAGTAAAGGTATTG | 160          | NM_022707        |
| ERbeta    | AGCCTTAGTCTCTCTGCTTC TGCTGGCGGAACACGTGAGTACG | 160          | XM_006240221.2   |
| GPR30     | TCTACCTAGTCCCTGTTG AGGCAGGAGGAAGAGAGAGAG  | 151          | NM_133573.1      |
| GAPDH     | CTCCATTTCTCCACCTTTG AGGGCTCTCTTCTTGCTCTC   | 157          | NG_028301        |