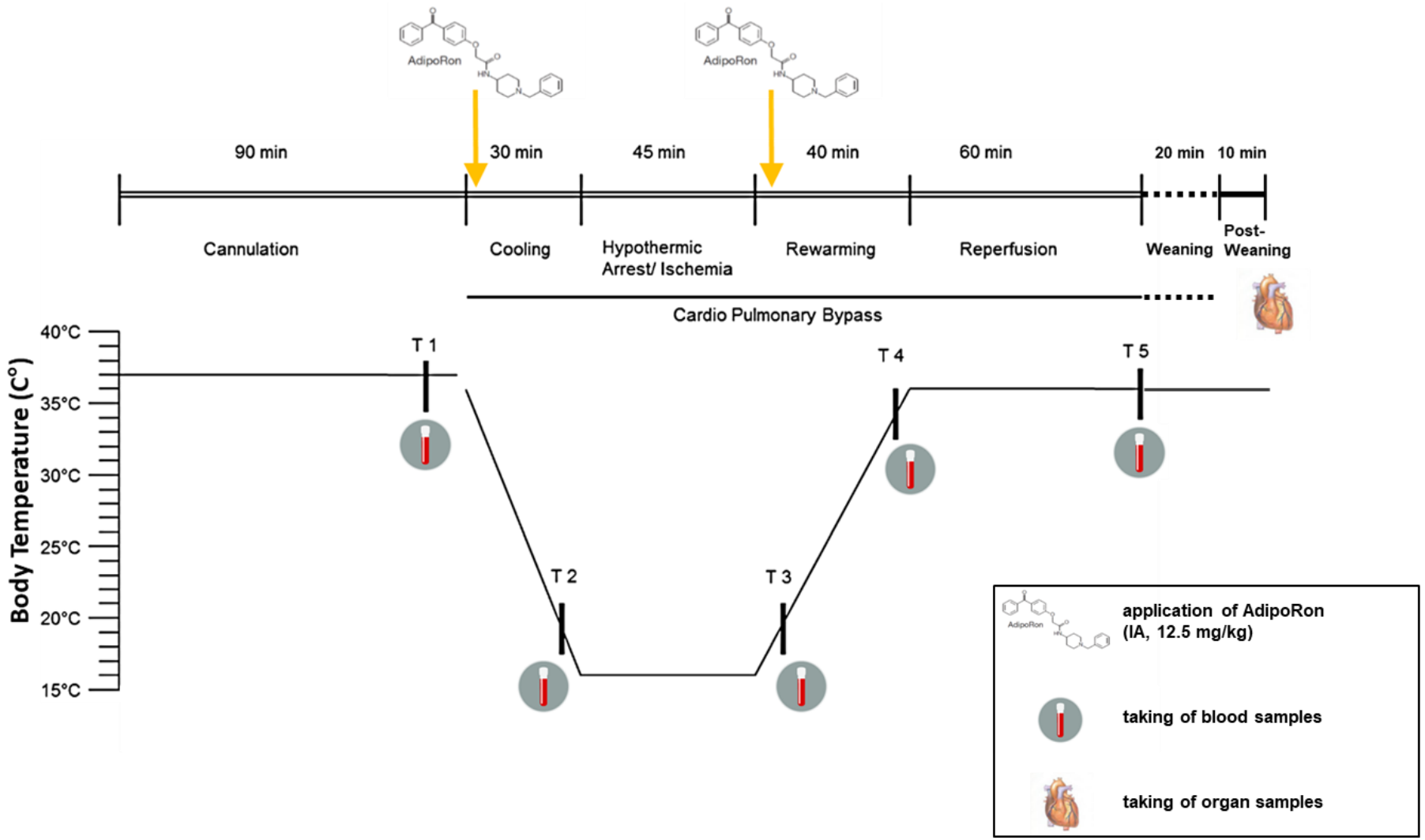
Supplementary Material

Data S1. **Supplemental Materials and Methods**

Quantitative real-time polymerase chain reaction (qPCR)

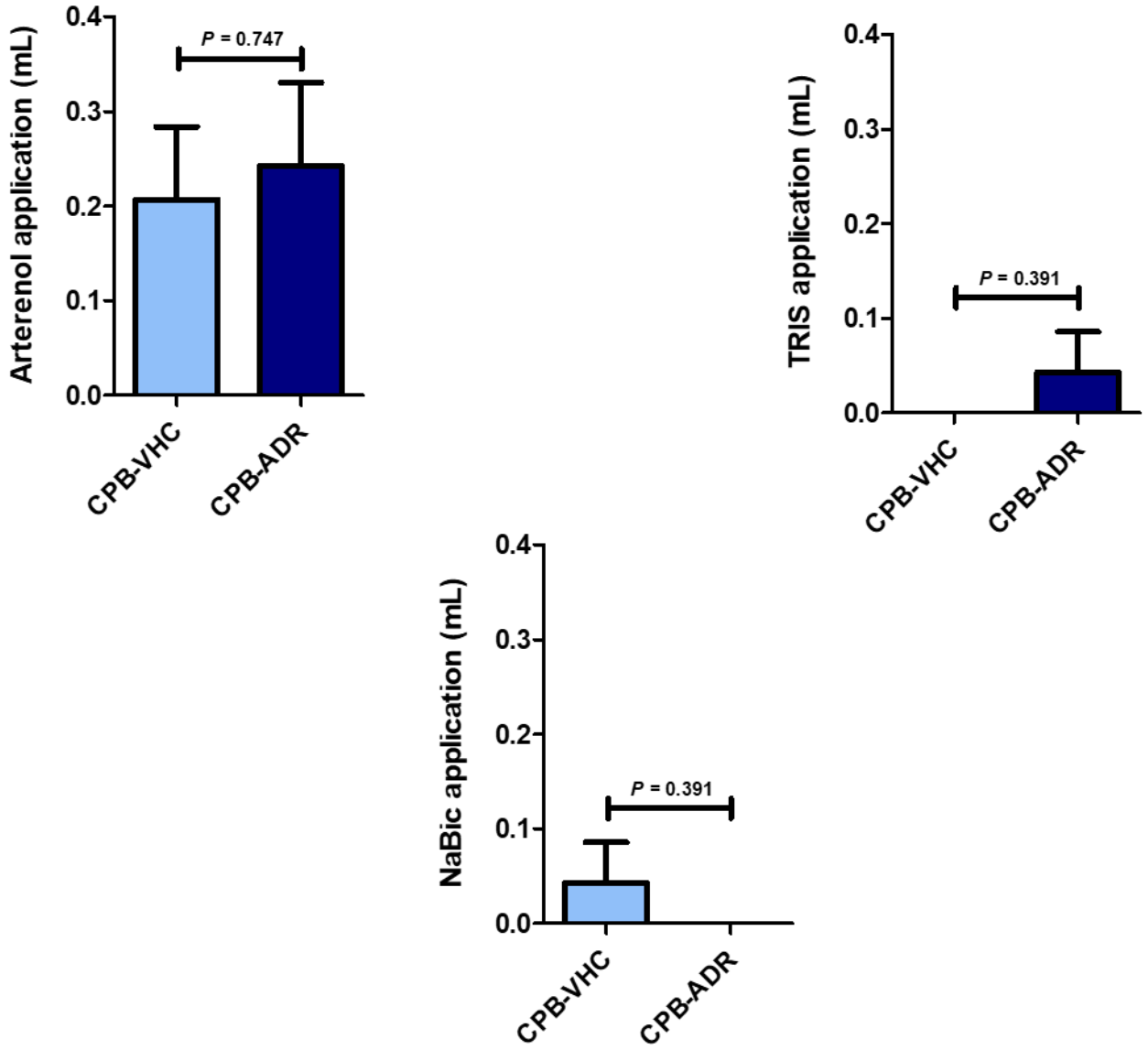
qPCR was performed using self-designed primers against mRNA transcripts encoding rat tumor necrosis factor α (TNF)-α (forward: GCTCCCTCTCATCAGTTCCA, reverse: GCTTGGTGGTTTGCTACGAC), interleukin (IL)-1β (forward: TCTTTGAAGAAGAGCCC GTCC, reverse: GCAGTGCAGCTGTCTAATGG), IL-10 (forward: GACGCTGTCATCGA-TTTCTCCC, reverse: GCCTTGTAGACACCTTTGTCTTG), C-C chemokine ligand (CCL)2 (forward: CTGTAGCATCCACGTGCTGT, reverse: GGTGCTGAAGTCCTTAGGGT), inducible nitric oxide synthase (iNOS) (forward: CTTGTTCAGCTACGCCTTCA, reverse: TGCCAAATGTGCTTGTAACC), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox2 subunit) (forward: TAGCACTTCACACGGCCATT, reverse: ATATGGGTCC GAAGTCCCGA), or hypoxanthine phosphoribosyltransferase (HPRT)1 (forward: GACCG GTTCTGTCATGTCG, reverse: ACCTGGTTCATCATCACTAATCAC), as well as human TNF-α (forward: CTGCTGCACTTTGGAGTGAT, reverse: GCCAGAGGGCTGATTAG AGA), IL-1β (forward: AGCTGATGGCCCTAAAC AGA, reverse: GGAGATTCGTAGCTG GATGC), IL-10 (forward: CCTGACCACGCTTTC TAGCT, reverse: GGCTCCCTGGTTTC TCTTCC), CCL2 (forward: GCTCATAGCAGCCACCTTCA, reverse: AGGTGACTGGGGC ATTGATT), iNOS (forward: AAGCAGCAGAATGAGTCCCC, reverse: TGCATCCAGCTT GACCAGAG), intercellular adhesion molecule (ICAM)-1 (forward: GCCAACCAATGTGCT ATTCA, reverse: AGTTCCACCCGTTCTGGAGT), vascular cell adhesion molecule (VCAM)-1 (forward: TGCGGGAGTATATGAATGTGAA, reverse: GCACGAGAAGCTCA GGAGAA), or 18S ribosomal RNA (rRNA) (forward: CAGCCACCCGAGATTGAGCA, reverse: TAGTAGCGACGGGCGGTGTG).

Figure S1. **Experimental time line of the cardiopulmonary bypass (CPB) procedure.**



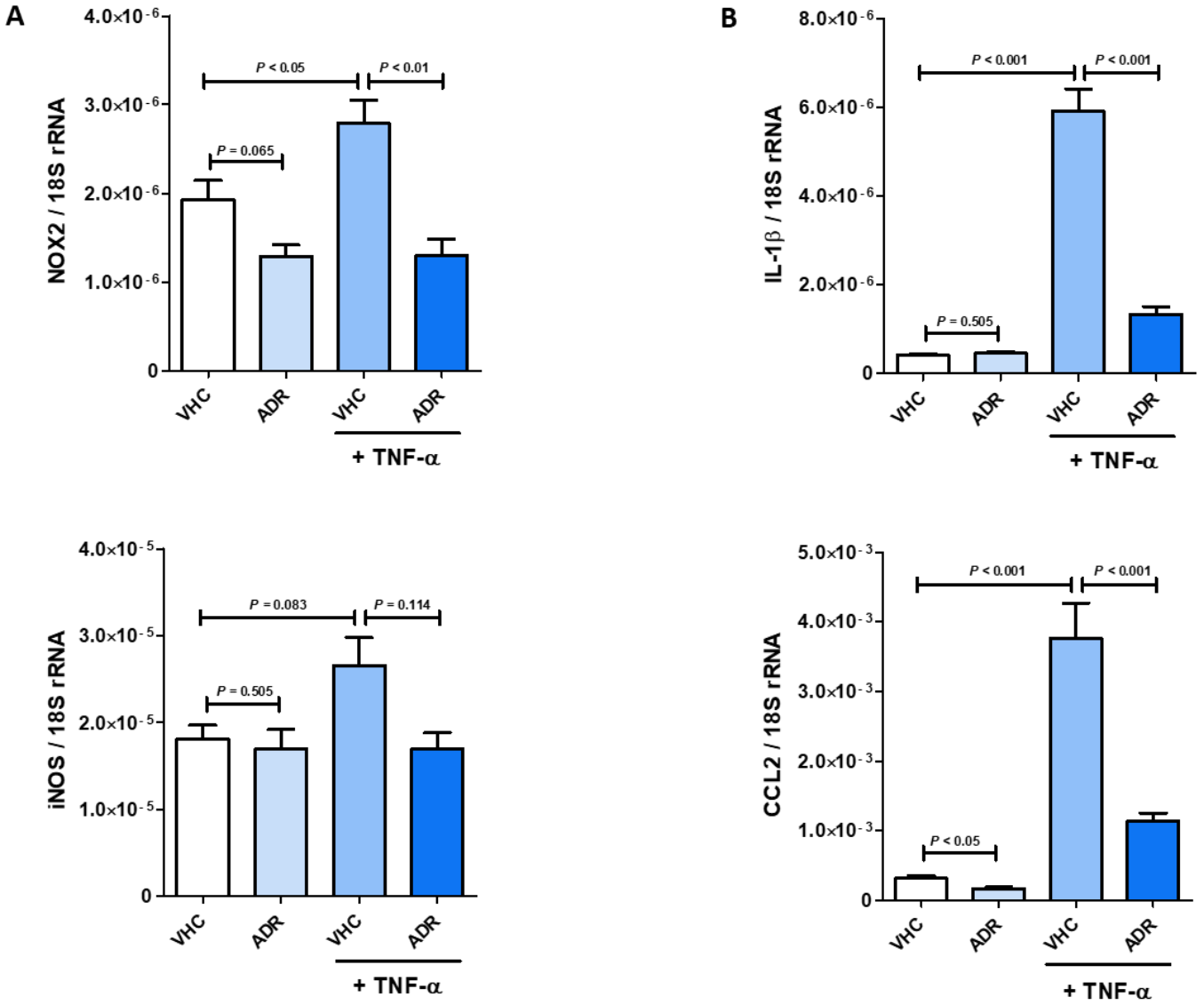
Male Wistar rats were cannulated, connected to a heart-lung machine (HLM) and cooled to a temperature of 16°C within 30 min before they underwent 45 min of deep hypothermic circulatory arrest (DHCA) with global ischemia. Restart of the CPB was followed by rewarming and 60 min of reperfusion. Subsequently, the rats were weaned from the CPB over a period of 20 min by stepwise ten percent reductions of the CPB flow rate performed every 2 min. After the weaning process was completed the animals were observed for a further 10 min and finally euthanized. Hemodynamic and vital parameters were recorded throughout the CPB procedure. Blood gas analysis was performed and blood as well as cardiac tissue samples were taken to measure systemic and organ-specific markers of inflammation, stress-response and injury. T1-T5 represent predefined time points for blood sampling, i.e. before CPB (T1), at 25°C in the cooling phase (T2), at 20°C (T3) and 35°C (T4) in the rewarming phase and after 60 min of reperfusion before the start of the weaning process (T5). AdipoRon (12.5 mg/kg body weight) or vehicle (dimethyl sulfoxide) was applied twice intra-arterial (IA) via the short cut access of the HLM at time points 10 min before the start of the CPB and with the beginning of the rewarming phase, respectively.

Figure S2. **AdipoRon does not affect compensatory drug application.**



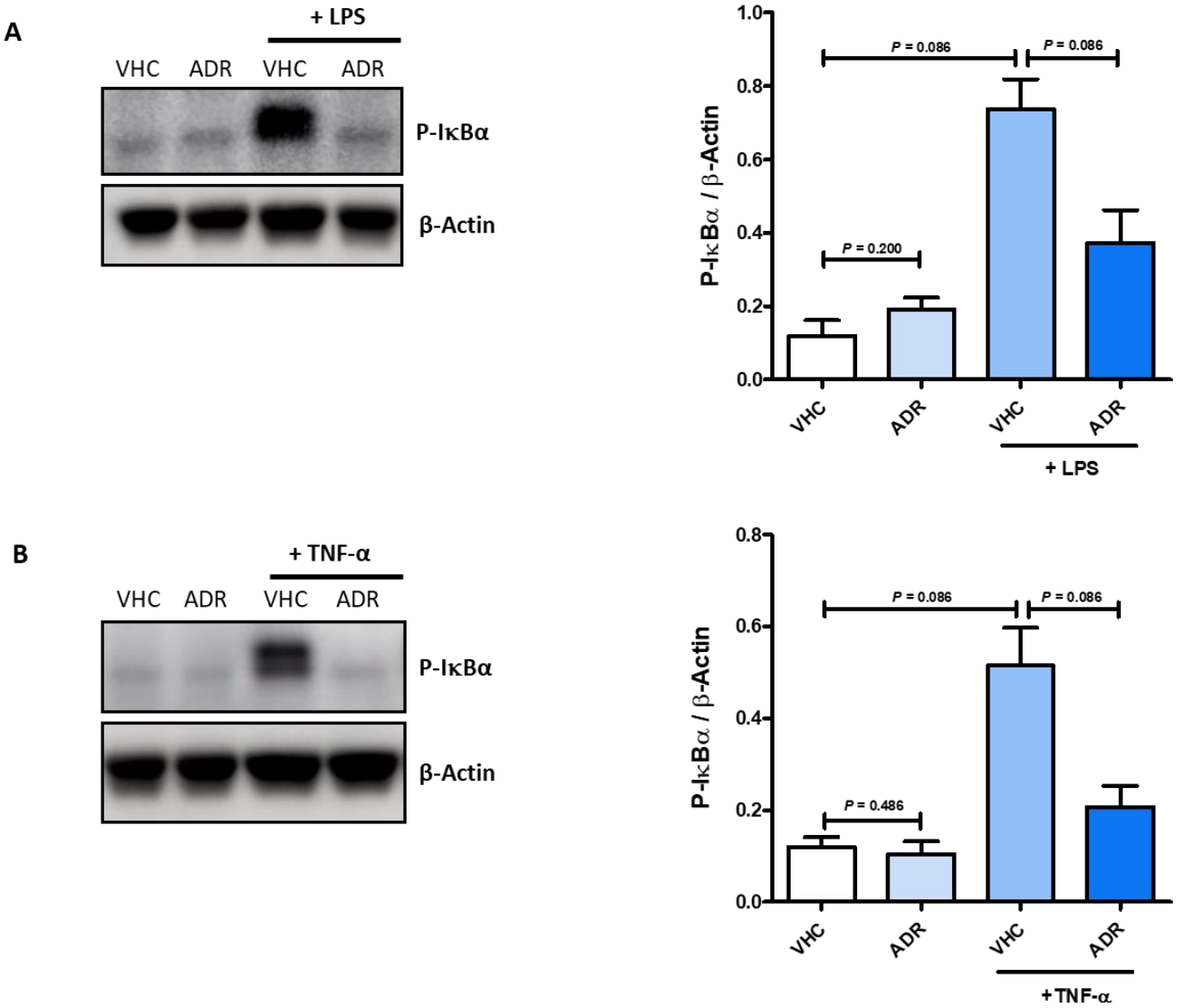
If necessary to maintain physiological vital parameters sodium bicarbonate (*NaBiC)*, trometamol (*TRIS*) or norepinephrine *(Arterenol)* were applied during the operative procedures of the cardiopulmonary bypass (CPB) model. Administered volumes of drugs were recorded for each animal. Results are presented as mean+SEM (n = 7 animals per group). Differences of applied drug volumes were analyzed statistically by comparing the experimental groups CPB-vehicle (VHC) and CPB-AdipoRon (ADR).

Figure S3. **AdipoRon inhibits the emergence of a systemic inflammatory response syndrome-associated tumor necrosis factor α (TNF-α)-induced inflammatory phenotype in cardiac myocytes.**



Cardiac myocytes were preincubated with AdipoRon (ADR, 80 µM) or vehicle (VHC, dimethyl sulfoxide) for 2 h before stimulation with TNF-α (10 ng/mL) for 1 h. mRNA expression of the (A) enzymes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox2 subunit) and inducible nitric oxide synthase (iNOS) as well as (B) the cytokine interleukin (IL)-1β and the chemokine C-C chemokine ligand (CCL)2 was measured relative to 18S ribosomal RNA (rRNA) by qPCR (n = 8). Results are presented as mean+SEM. Differences of marker expression levels between experimental groups were analyzed statistically by performing the indicated pairwise comparisons.

Figure S4. **AdipoRon attenuates systemic inflammatory response syndrome-associated Toll-like receptor 4 (TLR4) and (tumor necrosis factor α (TNF-α) signaling-induced phosphorylation of nuclear factor κB inhibitor α (IκBα) in cardiac myocytes.**



Cardiac myocytes were preincubated with AdipoRon (ADR, 80 µM) or vehicle (VHC, dimethyl sulfoxide) for 2 h before stimulation with (A) lipopolysaccharide (LPS, 1 mg/mL) for 1 h or with (B) TNF-α (10 ng/mL) for 15 min. The phosphorylation of IκBα in cell lysates was analyzed by immunoblot (n = 4). Left panel: Representative pictures of the resulting phosphorylated IκBα (P-IκBα, molecular weight: 40 kDa) and β-Actin (molecular weight: 45 kDa) band patterns. Right panel: Column bars indicate quantified P-IκBα/β-Actin expression ratios. Results are presented as mean+SEM. Differences of marker expression levels between experimental groups were analyzed statistically by performing the indicated pairwise comparisons.