

Mitochondrial Isolevuglandins Contribute to Vascular Oxidative Stress and Mitochondria-Targeted Scavenger of Isolevuglandins Reduces Mitochondrial Dysfunction and Hypertension

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Short title: Mitochondrial Isolevuglandins Contribute to Hypertension

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Representative HPLC chromatograms of MitoSOX treated HAEC

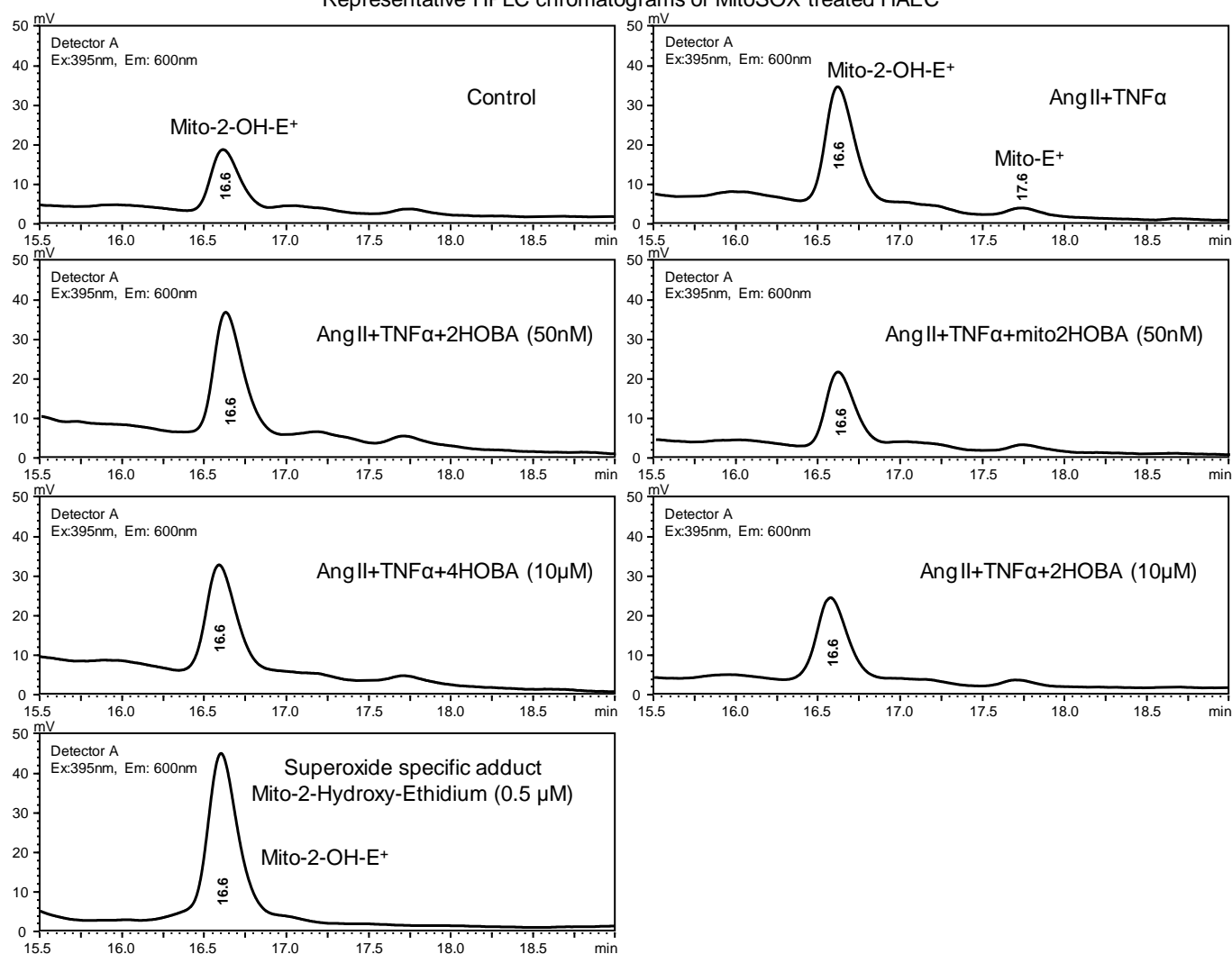


Figure S1. Representative HPLC chromatograms of MitoSOX treated human aortic endothelial cells (HAEC) using Betasil 5C18 column (Fisher Scientific). Cultured cells were supplemented with saline as a vehicle, mito2HOBA, 2HOBA or 4HOBA for 10 minutes prior to stimulation with angiotensin II and TNFα for 4-hours. Cells were washed out with KHB buffer and treated with MitoSOX (1 μM) for 30 minutes at 37 °C, and then collected into 300 μL methanol, homogenized and filtered for HPLC analysis. Mitochondrial superoxide was measured by accumulation of MitoSOX-superoxide specific product, mito-2-hydroxy-ethidium (Mito-2-OH-E⁺).⁴⁶

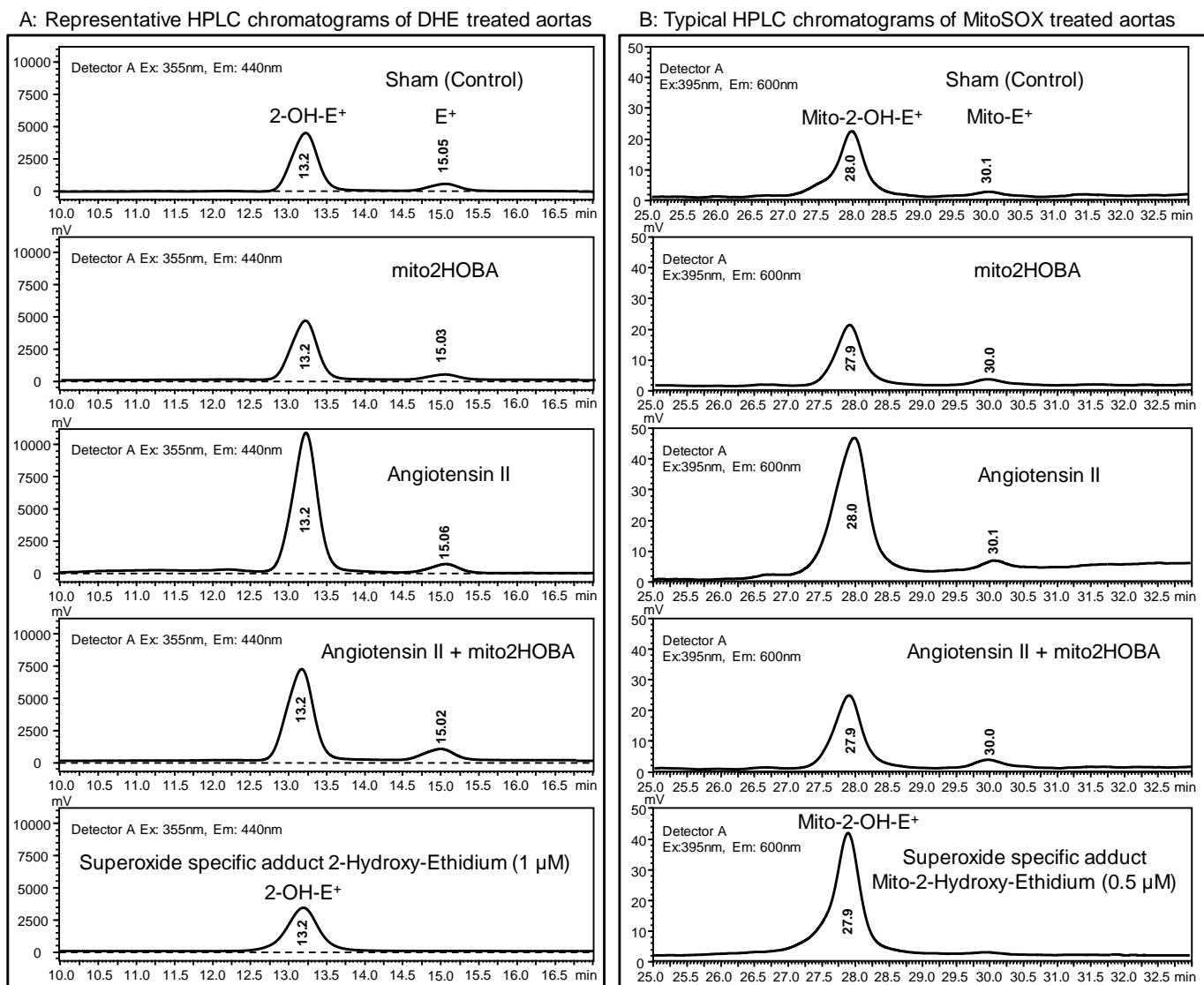


Figure S2. Representative HPLC chromatograms of DHE (A) and MitoSOX treated aortas using Nucleosil 5C18 column (Supelco). Aortas were isolated from Sham, angiotensin II-infused (0.7 mg/kg/day, 14-days) C57Bl/6J mice or animals supplemented with mito2HOBA in drinking water (0.1 g/L). Aortas were incubated with DHE (10 μM) or MitoSOX (1 μM) for 30 minutes at 37 °C, placed in 300 μL methanol, homogenized and filtered for HPLC analysis. Cellular superoxide was measured by accumulation of DHE-superoxide specific product, 2-hydroxy-ethidium (A). Mitochondrial superoxide was measured by accumulation of MitoSOX-superoxide specific product, mito-2-hydroxy-ethidium (B).⁴⁶

Representative ESR spectra of NO-Fe(DETC)₂ complexes in Fe(DETC)₂ treated aortas

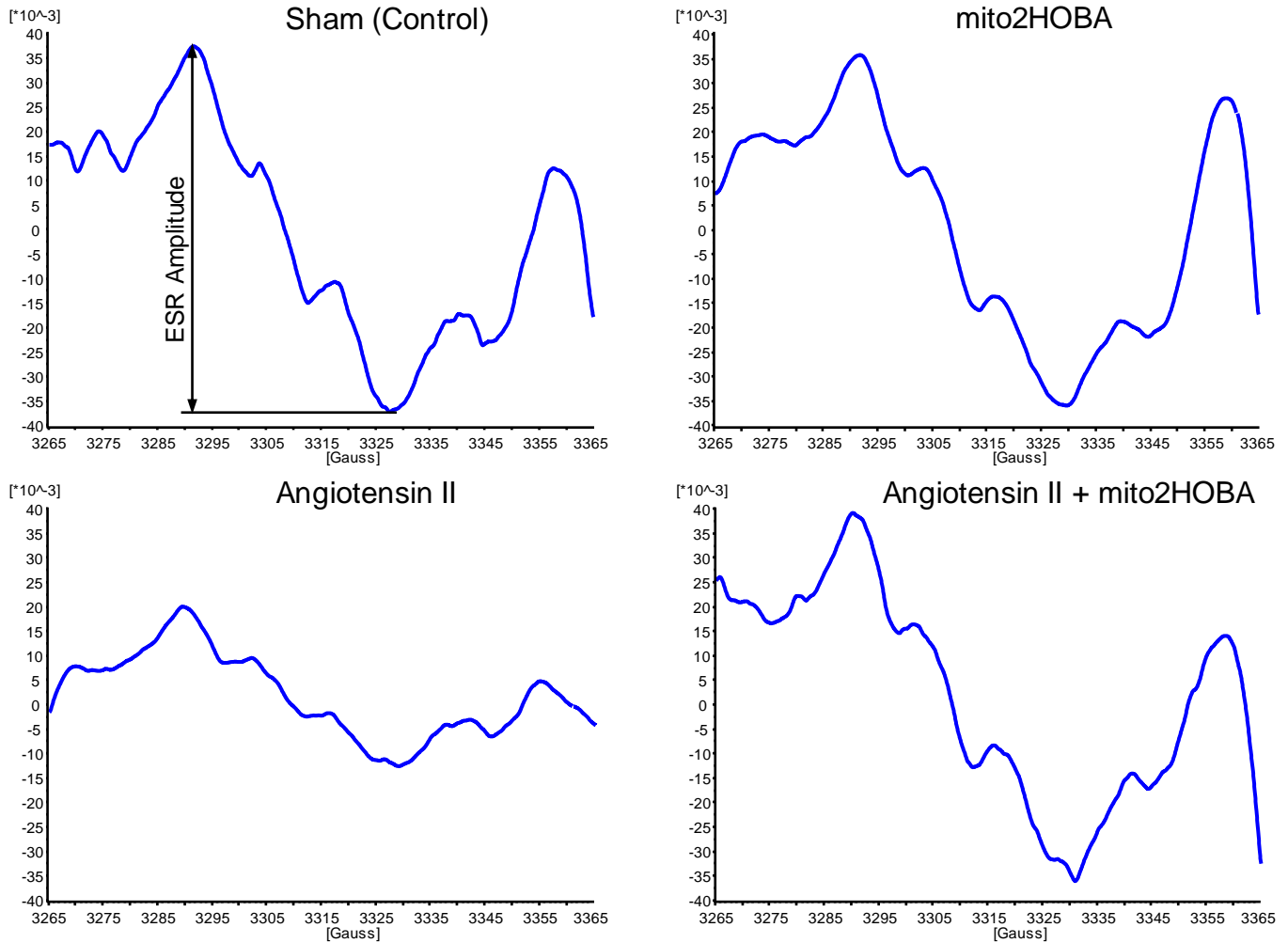


Figure S3. Representative ESR spectra aortas supplemented with NO-spin trap Fe(DETC)₂. Aortas were isolated from Sham, angiotensin II-infused (0.7 mg/kg/day, 14-days) C57Bl/6J mice or animals supplied with mito2HOBA in drinking water (0.1 g/L). Aortas were incubated with 250 μ M Fe(DETC)₂ for 60 minutes at 37 °C and then placed into 1 ml syringe and snap-frozen in liquid nitrogen. Production of endothelial nitric oxide was measured by accumulation of NO-Fe(DETC)₂ complex which has a specific ESR spectra.⁴⁷