

**Supplementary Figure 1. Heat exposure doesn’t increase NO levels in C2C12 myoblasts.** Cellular NO levels were determined by DAF-FM; values are % to control cells. Cells were treated with PBS (control) or 2 mM L-citrulline, then exposed to 37°C or 43°C for 2 h. L-NAME (1 mM) is a non-selective NO synthase inhibitor. n = 3 independent experiments and 30 cells were randomly selected for quantification in each group. Labeled means without a common letter differ, P < 0.05.

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**Supplementary Figure 2. Illustration shows L-citrulline regulates nitric oxide (NO)-mediated Drp1 inhibition and prevents heat-induced cell injury.** Heat stress causes mitochondrial fragmentation and dysfunction, which contribute to heat-induced skeletal muscle injury. L-citrulline increases cellular NO levels, which serves as a signal to decrease phosphorylation of Drp1 at Ser 616 (activation) and increases phosphorylation of Drp1 at Ser 637 (inactivation), resulting in fission inhibition. During heat stress, L-citrulline preserves mitochondrial morphology and function to protect myocytes from cell injury and apoptosis.