

Hyperthermia Promotes and Prevents Respiratory Epithelial Apoptosis  
through Distinct Mechanisms<sup>1</sup>

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ONLINE DATA SUPPLEMENT

### **Supplementary Figure Legends:**

#### **Figure S1. HS preconditioning protects against cell death induced by high dose**

**TNF $\alpha$  without concurrent HS** MLE15 cells were pre-conditioned with 2h 42°C HS beginning at the indicated time then incubated for 24h at 37°C with or without 8 ng/ml TNF $\alpha$  and survival was assessed by crystal violet staining. Mean  $\pm$  SE of four experiments. \* denotes  $p < 0.05$  vs. TNF $\alpha$  without HS preconditioning.

#### **Figure S2. I $\kappa$ B<sup>SR</sup> expression blocks nuclear translocation of NF $\kappa$ B p65.**

NF $\kappa$ B activation was compared in an MLE15 cell clone stably transfected with I $\kappa$ B<sup>SR</sup> and control MLE15 cells stably transfected with empty expression plasmid by treating with 2ng/ml TNF $\alpha$ , sequentially immunostaining for p65 (red), counterstaining with DAPI and imaging with fluorescent confocal microscopy to detect p65 nuclear translocation.

#### **Figure S3. HS preconditioning protects against cell death induced by Fas**

**activation and FRH.** MLE15 cells were pre-conditioned with 2h 42°C HS beginning at the indicated time then incubated with 2.5  $\mu$ g Jo2 anti-Fas for 24h at 37°C or 39.5°C and survival was assessed by crystal violet staining. Mean  $\pm$  SE of four experiments. † and ¶ denote  $p < 0.05$  vs 37°C and 39.5°C without HS, respectively.

#### **Figure S4. Pro-apoptotic effect of FRH persists when NF $\kappa$ B signaling is blocked.**

I $\kappa$ B<sup>SR</sup>-expressing MLE15 cells were treated with 2 ng/ml TNF $\alpha$  at 37°C or 39.5°C, sequentially lysed and immunoblotted for active caspase-3.

Figure S1

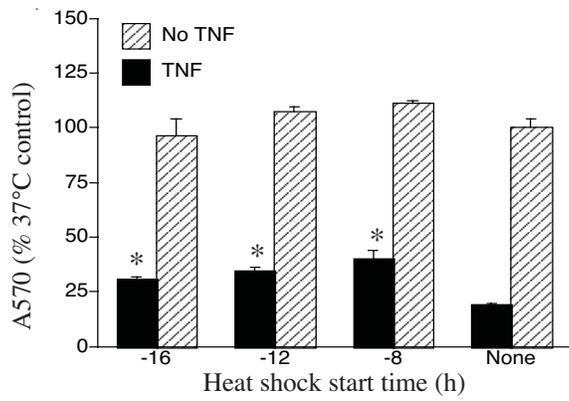


Figure S2

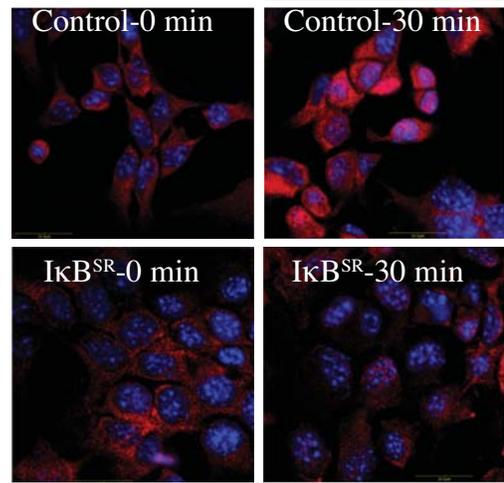


Figure S3

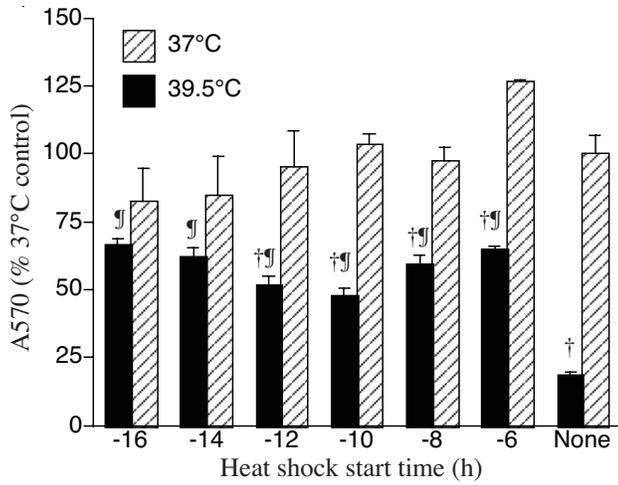


Figure S4

