ONLINE SUPPLEMENT

SUPPLEMENTARY METHODS

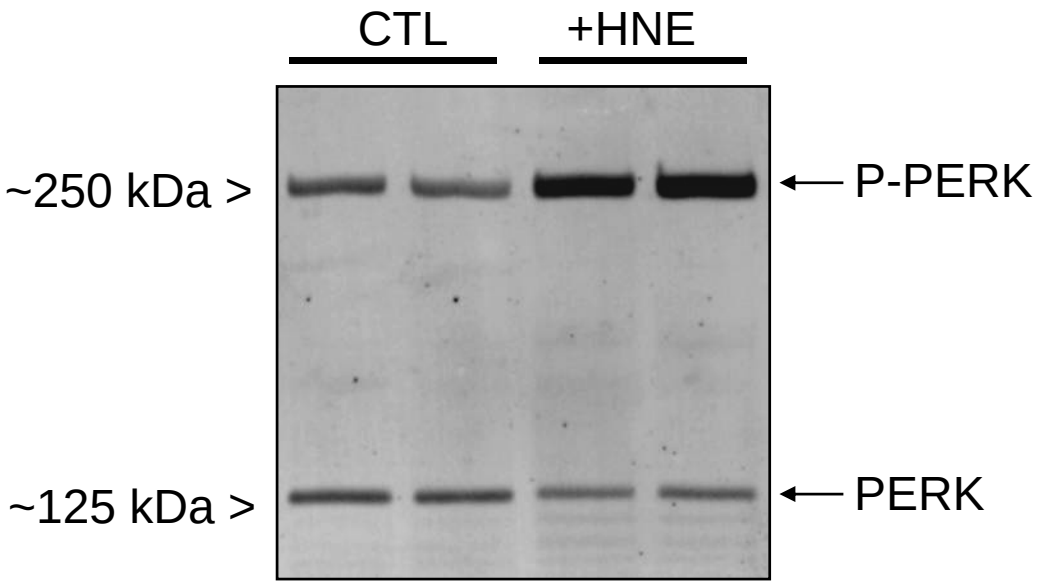
XBP-1 mRNA splicing: Total RNA was isolated using Trizol reagent (Sigma; St. Louis, MO). To detect whether IRE1α was active, PCR was used to amplify a 600-bp cDNA encompassing nucleotides 571-1144 of the *XBP-1* mRNA sequence. Digestion of the unprocessed *XBP-1* with the restriction enzyme *Pst* I normally yields fragments of ~300-bp. However, cleavage by active IRE1α of a 26-nt segment in *XBP-1* mRNA yields a 574-bp amplification product and removes the *Pst* I restriction site. Endogenous *XBP-1* mRNA was amplified by RT-PCR using the following primers: sense 5'-AAA CAG AGT AGC GCA GAC TGC-3'; antisense 5'-GGA TCT CTA AAA CTA GAG GCT TGG TG-3'. The PCR product was then digested with *Pst* I for 2 h at 37° C and detected on 2% agarose gels.

SUPPLEMENTARY FIGURE LEGENDS

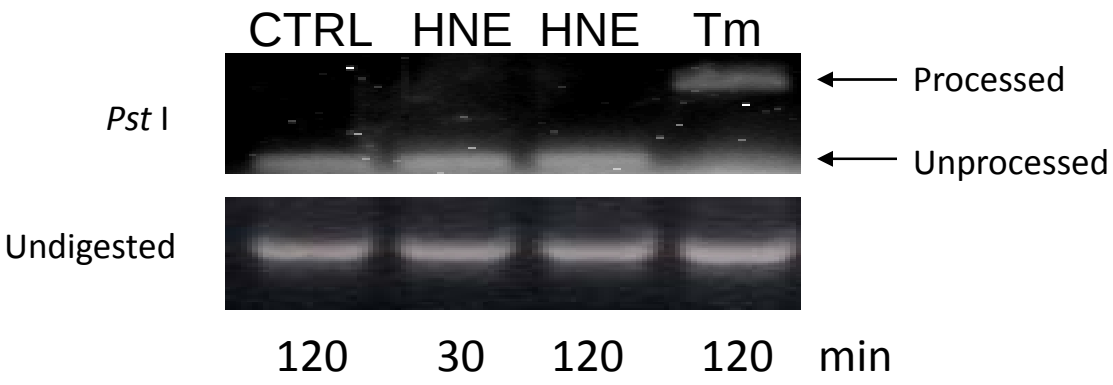
Suppl. Fig. 1. PERK phosphorylation in RASMC. Analysis of phospho-PERK (P-PERK) and PERK in RASMC treated with HNE: Cells were treated with HNE (50 μM) for 30 min and the lysates were collected for Western analysis with P-PERK and total PERK antibodies.

Suppl. Fig. 2. Activation of the IRE1α pathway, as detected by XBP-1 splicing, in RASMC. Cells were treated with vehicle (CTRL), HNE (50 μM), or tunicamycin (Tm; 10 μg/ml) for 30 min or 2 h, as indicated. After isolation of mRNA, the *XBP-1* mRNA product was amplified by PCR, digested with *Pst* I, and separated by electrophoresis. Loss of the *Pst* I restriction site is indicated by the processed form of *XBP-1* and suggests activation of the IRE1 pathway. Undigested PCR products were used as loading controls.

Suppl. Fig. 1



Suppl. Fig. 2


CTRL
HNE
HNE
Tm
Pst I
Undigested
Processed
Unprocessed
120
30
120
120
min