Supplemental Materials and Methods

Primary Cell Isolation/Culture

Type II alveolar epithelial cells were isolated from the lungs of C57BL/6 mice. Mice were anesthetized with pentobarbital and subsequently euthanized by exsanguination. The lungs were perfused with 10-20mL of PBS/EDTA until visually cleared of blood. The trachea was cannulated and tightly secured, after which the lungs were filled with 1mL of Dispase (BD Bioscience, San Jose, CA). 1 mL of 1% low melt agarose (Invitrogen, Carlsbad, CA) in water was then instilled into the lung. The lungs were removed and covered in crushed ice for 2 min, then placed in a sterile 15 mL tube with 2 mL dispase and incubated for 45 min at room temperature with gently mixing. The lungs cells were separated from the trachea and other connective tissue using blunt dissection forceps in a sterile petri dish. 10 mL of HAM's F-12 media (Invitrogen) with 0.01% DNase I (Sigma) were added to lung cells and swirled 5min at room temp. Lung cells were passed through a 100 micron filter, then through a 20 micron filter. Cell suspension was centrifuged at 130 x*g* at 8°C for 10 min and resuspended in 10 mL HAM'S F-12 with 1% Pen/Strep, Amphotericin, and 10 mM Hepes. The following antibodies (BD bioscience) were then added to the cell suspension: anti-CD16/32 at 0.65ug/million cells, anti-CD45 at 1.5ug/million cells), and anti-mouse TER-119/erythroid at 1ug/million cells. These were incubated at 37°C and 5% CO_2 for 30min, then centrifuged and resuspended in F-12 media. Streptavidin-coated magnetic beads (Pierce Biotechnologies, Rockford IL) were prepared by washing twice in PBS and once in the F-12 media, and 50 uL of bead suspension added to cell suspensions. These were incubated at room temperature 30 min with

rocking and then placed on magnetic separator 15 min. Cell suspension was carefully aspirated from beads, transferred to a new tube and centrifuged as above. Cells were then resuspended in 10 mL of HAM's F-12 with 1% Pen/Strep, Amphotericin, 15 mM HEPES, 0.8 mM $CaCl_2$, 0.25% BSA, 5 ug/mL insulin, 5 ug/mL transferrin, 5 ng/mL sodium selinite, and 2% FBS. Cells were transferred to petri dish and incubated overnight at 37°C and 5% CO_2. After 24hrs, unattached cells removed and centrifuged as above. Cells resuspended in 3mL of the F-12 media described above and plated onto collagen IV coated 96 well plates at 67,000 cells/well. After reaching near 100% confluency, cells were exposed to 95% O_2/5% CO_2 for 0, 24, 48, or 72 hours. RNA was isolated and qRT-PCR was performed using primer/probe assay to CHOP with HPRT as a normalizer as described previously.

Table S1: Oligonucleotide Sequences

Gene	Forward Sequence	Reverse Sequence
HPRT1	5'-GCAAACTTTGCTTTCCCTGGTTAAG-3'	5'-TCTGGCCTGTATCCAACACTTC-3'
HPRT1 Pobe	5'-CAGCCCCAAAATGGTTAAGGTTGCA-3'	
PKR	5'-CCGGTGCCTCTTTATTCAAA-3'	5'-ATCATTTTCCAGGGCTGTTG-3'
XBP-1	5'-TTACGGGAGAAAACTCACGGC-3'	5'-GGGTCCAACTTGTCCAGAATGC-3'

Figure S1 – Quantitative mRNA expression in isolated murine primary Type II lung epithelial cells of the alveolar epithelial gene, *Sftpc* (surfactant protein), and the fibroblast gene, *S100a4* (fibroblast-specific protein 1), normalized to *Hprt1*. Expression of these two genes in the murine alveolar epithelial cell line, MLE-12, and the murine fibroblast cell line, NIH3T3, are included as controls. Data are from three different cell isolations. Data are expressed as a relative quantity (RQ) as compared to MLE-12 expression for *Sftpc* and as compared to NIH3T3 expression for *S100a4*.

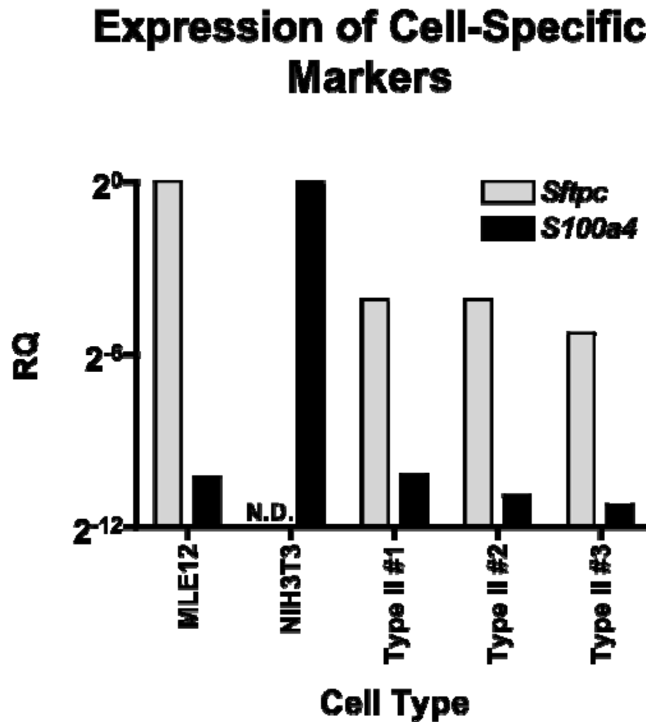

Figure S2 – Immunoblot of total PKR and actin from MLE12 cell lysates exposed to transfection reagent alone (untreated), control siRNA (cyclophilin B), or PKR siRNA for 72-hrs. Hyperoxia refers to cells exposed to 95% O_2 for the final 24-hrs. tm(+) – MLE-12 cells exposed to 5 μM tunicamycin for 24-hrs.

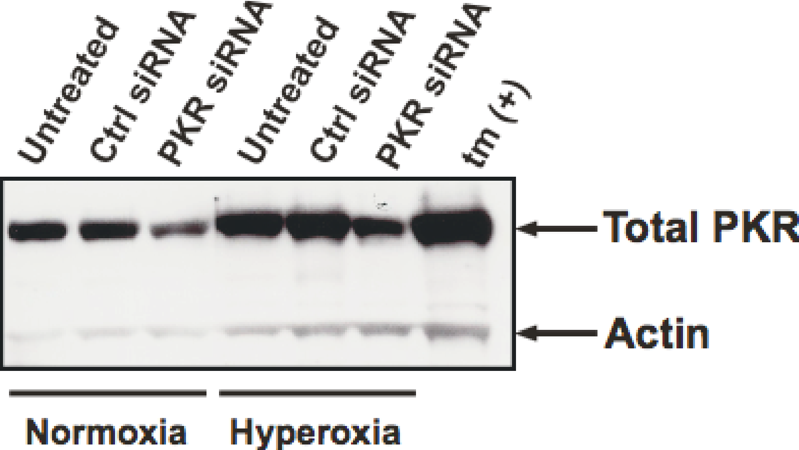

Figure S3 – Immunoblots for cleaved caspase-3 and actin in WT and *Ddit3-/-* mice exposed to normoxia or hyperoxia for 72-hrs. Expression of cleaved caspase-3 normalized to β-actin expression as measured by immunoblot densitometry, using ImageJ software.