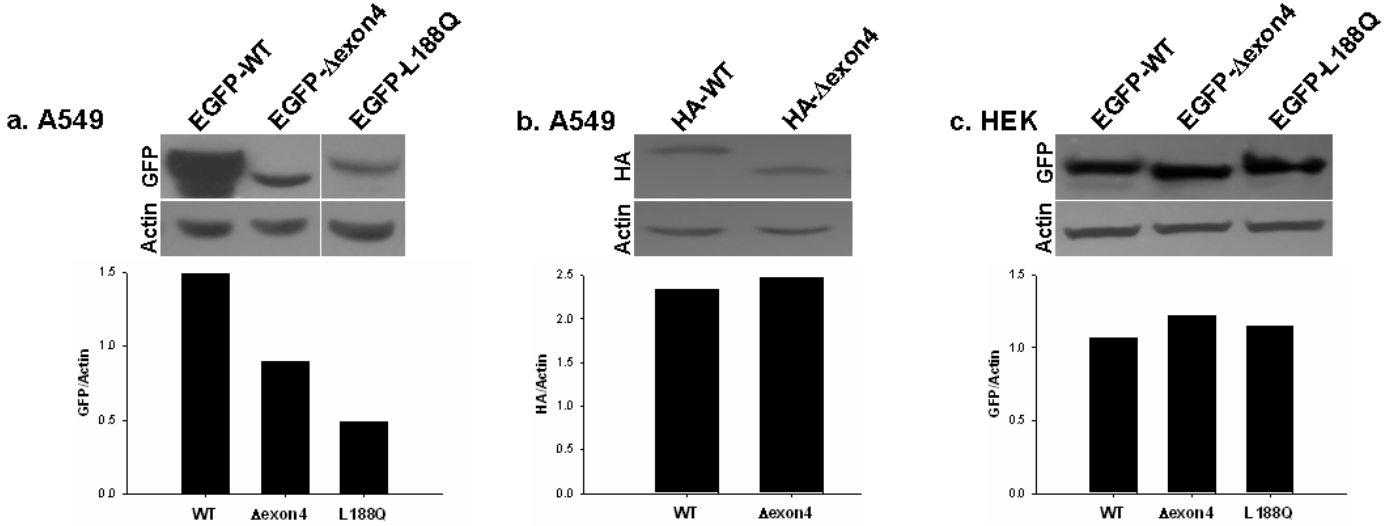
ENDOPLASMIC RETICULUM STRESS INDUCED BY SURFACTANT PROTEIN C BRICHOS MUTANTS PROMOTES PROINFLAMMATORY SIGNALING BY EPITHELIAL CELLS

Jean Ann Maguire, Ph.D., Surafel Mulugeta, Ph.D., and Michael F. Beers, M.D.

Surfactant Biology Laboratories
Pulmonary and Critical Care Division
Department of Medicine
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

SUPPLEMENTAL DATA FILE

Supplemental Figure 1



d.

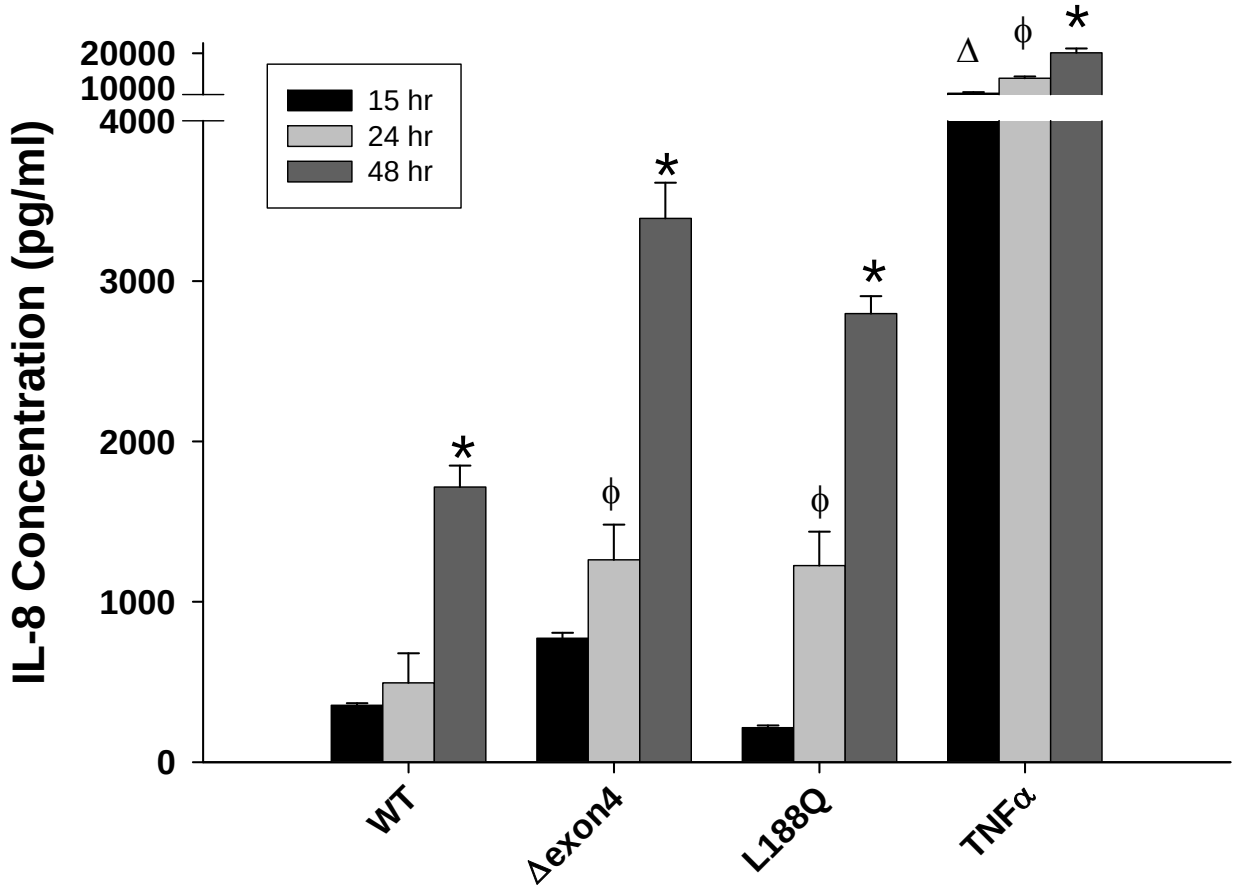
A549	% Transfected	S.D.
WT	12.3	2.6
Δexon4	8.2	2.2
L188Q	9.9	2.8

e.

HEK293	% Transfected	S.D.
WT	46.8	5.9
Δexon4	36.3	3.4
L188Q	45.7	1.9

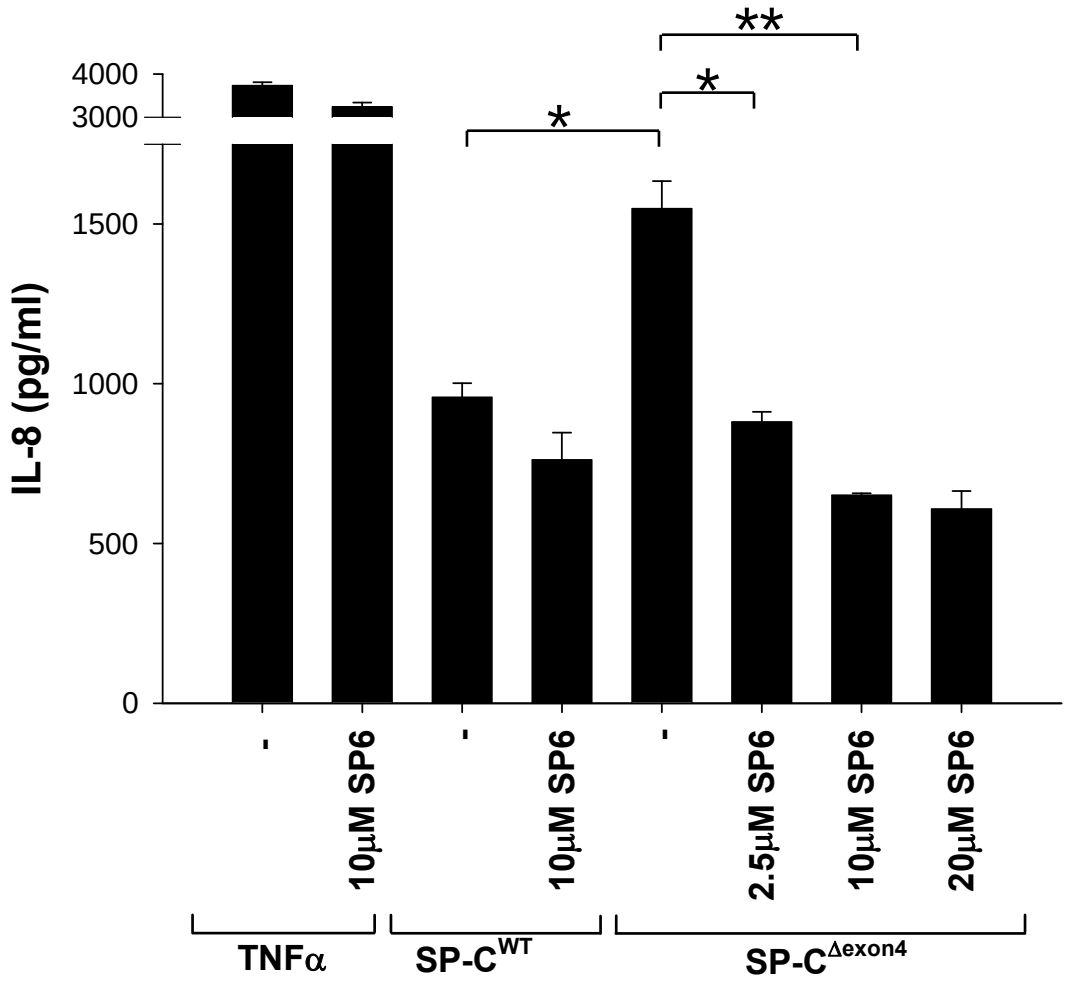
Supplemental Figure 1. Transfection Efficiency of SP-C Mutants in A549 and HEK293 Cell Lines. A549 (**a, b**) and HEK293 (**c**) cells were transiently transfected with the indicated SP-C constructs, and harvested 48 hours post-transfection. Cell lysates were analyzed for GFP expression, using a monoclonal GFP antibody as described in Methods. An anti-ß-actin antibody was used as a loading control. A representative immunoblot of each cell line is shown. Band intensities for the primary translation products were quantified by densitometry, and the ratio of GFP to ß-actin was determined. Transfection efficiency was also determined by manual counting of GFP positive cells in transiently transfected A549 (**d**) and HEK293 cells (**e**). The ratio of GFP-labeled to total cells was determined across 8 separate experiments, counting a minimum of 5 fields per condition. Results were averaged and expressed as a percentage of GFP positive cells.

Supplemental Figure 2



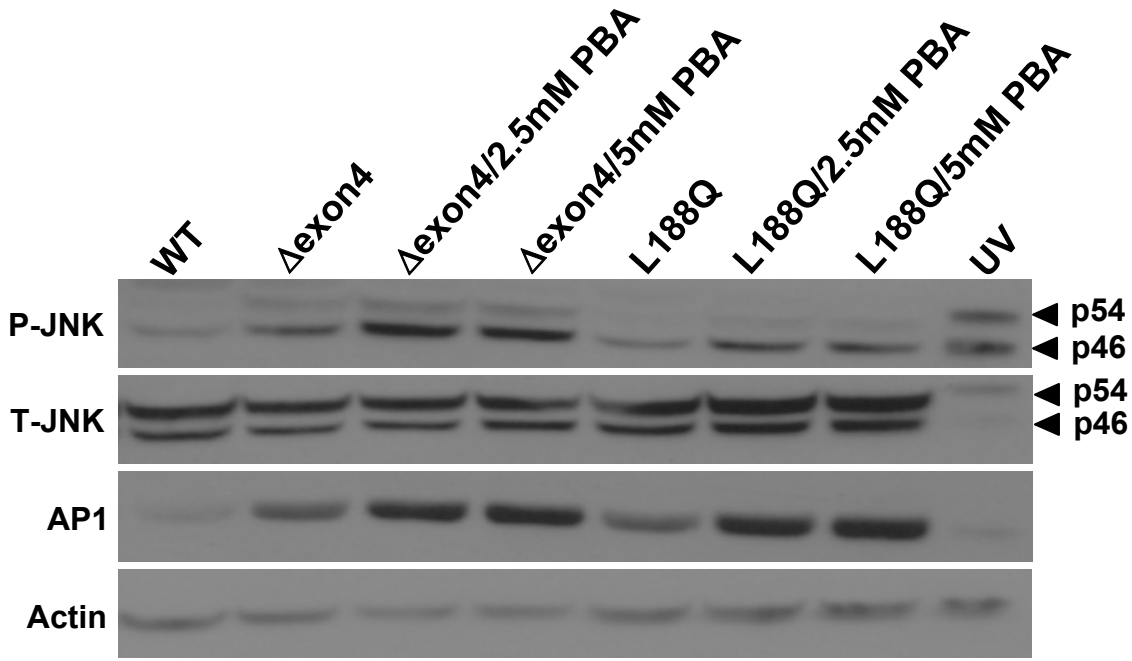
Supplemental Figure 2. **Time dependent Induction of IL-8 Release in SP-C BRICHOS Expressing Cells.** A549 cells were transiently transfected with the indicated GFP-tagged control, SP-C wild type, or mutant constructs using Lipofectamine 2000 reagent. Media was collected at 15, 24 and 48 hours post-transfection and IL-8 was measured by ELISA as described in *Methods*. In parallel, positive controls were generated for each of the 3 time points by treatment of untransfected cells with 10ng/ml TNFα 15 hrs prior to harvest. Data are expressed for IL-8 concentration in the media as Mean ± S.D. of triplicate samples. $\Delta p<0.05$ vs. 15 hour WT control, $*p<0.05$ vs. 24 hour WT control, and $\phi p<0.05$ vs. 48 hour WT control.

Supplemental Figure 3



Supplemental Figure 3. **Dose Response of IL-8 Release to SP600 125 Inhibitor.** A549 cells were transiently transfected with HA-tagged wild type or Δexon4 SP-C constructs using Lipofectamine 2000 reagent. Twenty-four hours post-transfection, the indicated concentration of SP600125 was added to specified samples. An overnight treatment of 10ng/ml TNFα was used as a positive control. Media was collected 48 hours post-transfection, and IL-8 was measured by ELISA as described in *Methods*. Data is expressed as the mean IL-8 concentration ± S.D. of triplicate samples.

Supplemental Figure 4



***Supplemental Figure 4*. 4-PBA increases JNK and AP1 activity in HEK cells expressing SP-C BRICHOS mutants.** HEK293 cells were transiently transfected with the indicated control or EGFP-SP-C constructs. Eight hours post-transfection, increasing concentrations of 4-PBA were added to SP-CΔexon4 transfected cells and harvested 48 hours post-transfection. UV treatment (40mJ/cm2) was used as a positive control. Cell lysates were analyzed for Phospho-SAPK/JNK, total SAPK/JNK, and Phospho-c-Jun (AP1) expression. An anti-ß-actin antibody was used as a loading control. Blots represent results from two experiments.