Supplementary Methods

Animal models of acute lung injury

Ischemia reperfusion. Adult C57BL/6 male mice were anesthetized with 4% isoflurane for 5 min in an anesthesia chamber and then with 2% isoflurane via nose cone prior to intubation by tracheostomy with a blunt 20-gauge needle. Intubated animals were connected to a mechanical ventilator (MiniVent, Harvard Apparatus, Holliston, MA) and mechanically ventilated with a tidal volume of 10 ml/kg, a rate of 150 breaths per minute, an end-expiratory pressure of 3-cmH_2O, an inspired oxygen fraction of 0.4, and 1.5% isoflurane. A subcutaneous injection of 1 ml D5LR was administered, and pancuronium (20 µl/gram body weight of a 0.1 µg/µl in D5LR) was given i.p. Pancuronium 10 µl/gram body weight was re-administered i.p. every 60 min for the duration of the experiment. Airway pressure and respiratory rate were continuously acquired (PowerLab, AD Instruments, Colorado Springs, CO). Mixed expired CO2 (CO_2SMO Model 7100, Novametrix, Wallingford, CT) was continuously monitored to confirm continued animal viability.

After collection of baseline data, isoflurane was increased to 2%, and the animals were placed in a right lateral decubitus position for left thoracotomy after prepping with alcohol. Left lung hila were isolated with minimal manipulation of the lungs, and microvascular clamps placed across all hilar structures (bronchus, artery, and vein). Thoracotomy sites were covered with moist surgical gauze, animals rotated back to supine position with isoflurane reduced to 1.5%, tidal volume decreased to 6 ml/kg and the respiratory rate increased to maintain expired CO_2 at a similar level.

After 60-min, left hilar clamps were removed, tidal volumes increased to 10 ml/kg, and respiratory rates decreased to 150 breaths/minute. Left lung was recruited by placing the lung on 15-cmH_2O for 30-sec. Mechanical ventilation was continued for an additional four hours of reperfusion after which the mouse was deeply anesthetized with isoflurane and euthanized by cardiac puncture and exsanguination. Right hila were clamped and left lungs were lavaged with

three 0.4 ml aliquots of PBS containing 0.6 mM EDTA. BALF was centrifuged at 1000 × *g* for 10-min. Supernatant was stored at –80ºC until proteomic analysis.

Staphylococcus aureus pneumonia. *S. aureus* was originally isolated from a patient with catheter-related sepsis and cultured as previously described (21). Adult C57BL/6 male mice were weighed and anesthetized with 4% isoflurane for 3-min and instilled with 2×10^7 cfu of *S. aureus* in 50 µl PBS, euthanized 24 h post-infection and right lung was lavaged with three 0.5 ml aliquots of PBS containing 0.6 mM EDTA at 37°C. BALF was spun at 1000 × *g* for 10-min at 4°C, and the supernatant collected and stored at –80°C for subsequent proteomic analysis.

Influenza pneumonia. A C57BL/6 adult male mice were weighed and anesthetized with 4% isoflurane for 3-min. The anesthetized animals were suspended by the front teeth, and 4,000 pfu of A/PR8/34 (mouse-adapted H1N1 influenza strain) in 50 µl PBS was deposited in the oropharynx and observed until aspirated. The animals returned to their cages and monitored daily. At 120-hr post-infection, the animals were euthanized and BALF obtained and processed identically to the *S. aureus* pneumonia protocol.

Shotgun proteomics analysis

Pooled BALF samples from humans and mice reduced, alkylated, tryptically digested, separated using 2D liquid chromatography followed by tandem MS/MS analysis. BALF proteins were identified using SEQUEST search engine followed by PeptideProphet and ProteinProphet (Figure 1).

Sample preparation. Equal volumes of each cell-free BALF supernatant sample were denatured with 8 M Urea in 50 mM NH_4HCO_3 then reduced by addition of 5 mM Tris(2-carboxyethyl) phosphine hydrochloride at 37°C for 30 min and alkylated by 10 mM iodoacetamide in the dark for 1hr. Alkylation was stopped by addition of 10 mM dithiothreitol for 5 min. The solution was diluted to 2 M Urea with 20% methanol/50 mM NH_4HCO_3, pH 8.5 and then underwent proteolysis for 16 hr using a 1:20 w/w trypsin-to-protein ratio. The tryptic digest was desalted with micro-spin cartridge (Nest Group Inc, MA) and completely dried using a

Speed-Vac (Thermo-Savant, Milford, MA). At this point, the sample was used directly for LC-MS/MS analysis.

LC-MS/MS Analysis. Each sample was analyzed by LCMS using a Michrom Paradigm MS4B HPLC system (Michrome Bioresources, CA) that was coupled as previously described [1] via electrospray ionization (ESI) on-line to a linear ion trap (LTQ) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (i.e. LTQ-FT ThermoFinnigan, CA). Peptides were loaded on a 100 µm i.d. × 18 mm long precolumn packed with 200 Å (5 *í*m) Magic C18 particles (C18AQ; Michrom) at a flow rate of 8ul/min for 5 minutes. Reversed-phase separations were performed using 75 mm i.d. × 360 um o.d. × 12 cm long fused silica capillary columns with a ESI frit (Polymicro Technologies, Phoenix, AZ), which were slurry-packed in-house with 5 mm, 200 Å pore size Magic C-18 AQ beads (Michrome Bioresources, CA) in a pressure cell (Brechbuhler, Spring TX) at a flow rate of 60ul/min. After injecting 0.1 µg of total sample onto the column, a 5 min wash was applied and peptides were eluted using a linear gradient of 5% solvent B (100% acetonitrile) to 35% solvent B in 60 min, then to 80% in 5 min where the acetonitrile concentration remained for 10 min prior to column re-equilibration at 5% for 15 minutes. Buffer C remained constant at 10% during gradient operation. The LTQ-FT mass spectrometer was operated in a data-dependent mode such that selected precursor ion survey scans were acquired in the FT-ICR with resolution R=100,000 at m/z 524 (after accumulation to a target value of 1,000,000 in the ICR cell) and peptide tandem mass spectra in the LTQ ion trap. The five most intense ions were sequentially isolated in the linear ion trap and subjected to collision induced dissociation (CID) in series using a trap target value of 5,000 and 60 sec of dynamic exclusion. The total MS-MS/MS scan cycle time was ~1.5s. The general mass spectrometric conditions were: ESI voltage, 1.3 kV; ion transfer tube temperature, 200 ºC; and normalized collision energy, 35%. Ion selection threshold, 3000 counts for tandem MS. Precursor ions were selected over the entire m/z range (350-2000). Then, the experiment was repeated using gas phase fraction (*e.g.* precursor ions were selected over a limited *m/z* range

during each injection). The following six *m/z* range were applied: 350-550, 500-700, 650-850, 800-1000, 950-1500, and 1450-2000.

Protein Identification. For database searches, tandem mass spectral RAW (ThermoFinnigan) files were first converted to mzXML format using ReAdW software program [2]. The individual spectra for each RAW file were extracted from the mzXML file into corresponding DTA files (http://tools.proteomecenter.org/mzxml2other.php). The tandem mass spectra were then matched to protein sequence in the IPI Human and Mouse database (http://www.ebi.ac.uk/IPI) using SEQUEST. Criteria for matching a peptide tandem mass spectrum to a peptide sequence were: Xcorr >1.9 with charge state 1+, Xcorr>2.2 with charge state 2+, or Xcorr>3.75 with charge state 3+, as well as ΔCn >0.1. Peptide tandem mass spectra passing these criteria were utilized for protein identifications. A protein was considered to be identified only if ProteinProphet probability > 0.8 [3] and if more than one unique tryptic peptide was found for each protein.

References

1. Yi EC, Lee H, Aebersold R, Goodlett DR. A microcapillary trap cartridge-microcapillary high-performance liquid chromatography electrospray ionization emitter device capable of peptide tandem mass spectrometry at the attomole level on an ion trap mass spectrometer with automated routine operation. *Rapid Commun Mass Spectrom* 2003: 17(18): 2093-2098.
2. Pedrioli PG, Eng JK, Hubley R, Vogelzang M, Deutsch EW, Raught B, Pratt B, Nilsson E, Angeletti RH, Apweiler R, Cheung K, Costello CE, Hermjakob H, Huang S, Julian RK, Kapp E, McComb ME, Oliver SG, Omenn G, Paton NW, Simpson R, Smith R, Taylor CF, Zhu W, Aebersold R. A common open representation of mass spectrometry data and its application to proteomics research. *Nat Biotechnol* 2004: 22(11): 1459-1466.
3. Nesvizhskii AI, Keller A, Kolker E, Aebersold R. A statistical model for identifying proteins by tandem mass spectrometry. *Anal Chem* 2003: 75(17): 4646-4658.