Online Supplementary Material

Animal studies. All animal care and treatment procedures were approved by the University of Chicago Institutional Animal Care and Use Committee. Animals were handled according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Bacterial lipopolysaccharide (LPS, 0.63 mg/kg body wt; Escherichia coli O55:B5) or sterile water was injected intratracheally in a small volume (20-30 µl) using a 20-gauge catheter (Exelint International, Los Angeles, CA). Iloprost (20 µg/kg), BrcAMP (20 µg/kg), or sterile saline solution were administrated two times, concurrently and after 90 min of LPS instillation by intravenous injection in the external jugular vein. These doses have been selected based on results of pilot studies, which showed potent anti-inflammatory and barrier protective effects of iloprost and Br-cAMP without visible adverse effects on experimental animals. These concentrations are well below the maximal concentrations (iloprost - up to 200 µg/Kg, [1]) and Br-cAMP - up to 10 mg/Kg, [2]) used in vivo by other groups. After 16 hours, animals were sacrificed by exsanguination under anesthesia. Bronchoalveolar lavage (BAL) was performed using 1 ml of sterile Hanks balanced salt buffer and measurements of cell count, protein concentration, and myeloperoxidase activity were conducted as previously described [3]. Evans blue dye (30 ml/kg) was injected into the external jugular vein 2 hours before termination of ventilation to assess vascular leak as described previously [4].

References:

1. Zhu Y, Liu Y, Zhou W, Xiang R, Jiang L, Huang K, Xiao Y, Guo Z, Gao J. A prostacyclin analogue, iloprost, protects from bleomycin-induced pulmonary fibrosis in mice. *Respir Res* 2010: 11: 34.

2. Irie K, Fujii E, Ishida H, Wada K, Suganuma T, Nishikori T, Yoshioka T, Muraki T. Inhibitory effects of cyclic AMP elevating agents on lipopolysaccharide (LPS)-induced microvascular permeability change in mouse skin. *Br J Pharmacol* 2001: 133(2): 237-242.

3. Fu P, Birukova AA, Xing J, Sammani S, Murley JS, Garcia JG, Grdina DJ, Birukov KG. Amifostine reduces lung vascular permeability via suppression of inflammatory signalling. *Eur Respir J* 2009: 33(3): 612-624.

4. Birukova AA, Fu P, Xing J, Yakubov B, Cokic I, Birukov KG. Mechanotransduction by GEF-H1 as a novel mechanism of ventilator-induced vascular endothelial permeability. *Am J Physiol Lung Cell Mol Physiol* 2010: 298(6): L837-848.