#### SUPPLEMENTAL MATERIAL

### **Supplemental Methods**

### **Gene expression**

Total RNA was extracted using either TRIzol (Invitrogen, Carlbad, CA or RNeasy kit (Qiagen) according to the manufacturer's instructions and differences in gene expression were determined using Real-Time RT-PCR as previously described<sup>1</sup>. Gene expression was determined using comparative threshold cycle ( $\Delta\Delta C_T$ ) as suggested by the manufacturer (Applied Biosystems, Foster City, CA) normalizing each sample to 18s rRNA. The following primer sets were purchased from Assays on Demand (Applied Biosystems): 18s rRNA (cat #4310893E) and *SOD3* (cat# Mm01213380-s1).

### Proinflammatory cytokine and chemokine measurements

Cytokine and chemokine concentration in mouse lung tissue were measured using the MSD (MesoScale Discovery, Gaithersburg, MD) Proinflammatory Panel 1 V-PLEX kit.

#### Total protein in bronchoalveolar lavage

Total protein was quantitated using Coomassie Plus (Bradford) Assay kit (Thermo Fisher Scientific, Rockford, IL) per manufacturer's instructions.

## **Supplemental Figures**

#### **Figure legends**

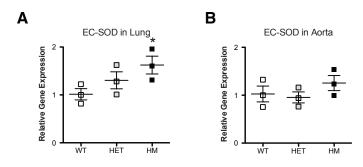
**Fig 1.** (A) Expression of *SOD3* was measured by Real-Time RT-PCR in whole lung tissue from WT (n = 3), HET (n = 3) and HM (n = 3) animals. Gene expression was normalized to 18s rRNA

expression and displayed relative to WT samples. (**B**) Expression of *SOD3* was measured by Real-Time TR-PCR in isolated aortas from WT (n = 3), HET (n = 3) and HM (n = 3) animals. Gene expression was normalized to 18s rRNA expression and displayed relative to WT samples. (**C**) Proinflammatory cytokines and chemokines in lung tissue from WT and R213G animals at 0 (WT n = 4, R213G n = 3), 4 (WT n = 8, R213G n = 4) and 24 hours (WT n =9, R213G n = 4) post LPS inhalation. Mediators are expressed as pg per 30ug of total protein. (**D**) Total protein in broncoalveolar lavage fluid (BALF) in WT and R213G animals at 0 (WT n = 3, R213G n = 2), 4 (WT n = 7, R213G n = 4) and 24 hours (WT n = 4, R213G n = 7) post LPS inhalation. Data are presented as mean  $\pm$  S.E. # P < 0.05 compared to samples at 0 hours for each genotype. \* P <0.05 comparing WT to R213G samples under identical conditions.

## **Supplemental References**

1. Hartney JM, Brown J, Chu HW, Chang LY, Pelanda R, Torres RM. Arhgef1 regulates alpha5beta1 integrin-mediated matrix metalloproteinase expression and is required for homeostatic lung immunity. *The American journal of pathology*. 2010;176:1157-1168

# **Supplemental Figure 1**



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