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[1]. 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 ± 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