Intratracheal Administration of Acat-1 Inhibitor K-604 Reduces Pulmonary Inflammation Following Bleomycin-induced Lung Injury

Emily R. Stevenson¹, Melissa L. Wilkinson¹, Elena Abramova¹, Changjiang Guo¹, Andrew J. Gow¹*

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Supplemental Data

Supplemental Fig 1. Flow cytometric analysis of alveolar macrophages in the BAL fluid. Cells were isolated from the BAL 7d post-ITB or PBS administration, ± intratracheal K-604
administration. Cells were collected and stained with the fluorescent antibodies listed in Table 1. Cells were analyzed using a Gallios 10-color flow cytometer (Beckman Coulter, Brea, CA). Using Kaluza software (Beckman Coulter, Brea, CA) cells were gated upon size and complexity (A, B), CD45 positivity (C) and viability (D). Cells that were positively stained for both Siglec F and F4/80 (E) were determined to be alveolar macrophages (AMs), highlighted in the green square. AMs were then analyzed for CD11c and CD11b expression (F) and further characterized (Fig 5).
Supplemental Fig 2. Flow cytometric analysis of interstitial macrophages from lung tissue digest. Right lung lobes from treated animals were digested and immunomagnetically separated based upon the presence of CD45 (Section 2.5). CD45+ cells were isolated and stained with the fluorescent antibodies listed in Table 1. Cells were analyzed using a Gallios 10-color flow cytometer (Beckman Coulter, Brea, CA). Using Kaluza software (Beckman Coulter, Brea, CA) cells were gated upon size and complexity (A, B), CD45 positivity (C) and viability (D). Cells that stained positively for F4/80 and exhibited absence of Siglec F (E) were then analyzed for CD11b expression (F). These cells were categorized as interstitial macrophages for further analyses (Fig 6).