Online Data Supplement

ADIPOSE STEM CELL TREATMENT IN MICE ATTENUATES LUNG AND SYSTEMIC INJURY INDUCED BY CIGARETTE SMOKING

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Figure E1. **ASCs home to mouse lungs following systemic injection.** **A**. ASC were intravenously delivered into ApoE mice and assessed for B-galactosidase expression at 1hr in fresh lungs. Note the vascular distribution of the blue-stained ASC (arrows). **B**. Biochemical quantification of lung ASC by immunoblotting with a specific GFP antibody of lung lysates obtained at day 3 following the intravenous injection of GFP-labeled ASC into NS2 mice that were also treated with a VEGFR inhibitor SU5416 (20mg/kg) or its vehicle (-). Vinculin was used as a loading control. **C-D.** Localization of GFP-expressing murine ASC (brown, arrow) on lung sections following fixation and immune staining with GFP antibody (C, D) and counterstaining with hematoxylin (C). Lungs of DBA/2J mice were harvested 7 days following ASC administration (3×10^5). Note (arrows) the presence of ASC intercalated among the bronchial epithelial layer (C) and in the lung parenchyma (D). Barsize 100 μm. **E.** Flow cytometry panels showing detection of events consistent with Di-I-labeled murine ASC in lung homogenates of DBA2 mice, 21 days following ASC intravenous injection, compared to control littermate mice, which did not receive ASC. Note in red, fluorescent cells comprising approximately 4% of total cell population, in the ASC-injected mice.

Figure E2. ASC treatment modulates the CS-induced activation of MAPK and AKT signaling pathways. **A-C**. Levels of p38 MAPK, JNK1, and Akt activation measured by densitometry of phosphorylated proteins relative to total levels of respective proteins detected by immunoblotting of total lung homogenates with specific antibodies. The lungs from DBA/2J mice were harvested following 4 months of air or CS exposure. A third group was treated with ASC (3×10^5 cells per injection, injected intravenously

every other week), during the month 3 and 4 of CS exposure (mean + SEM; n=4-6 lung samples from individual mice; *p<0.05 versus air control; #p<0.05 versus CS; ANOVA).

Figure E3. ASC attenuate the weight loss caused by CS. Representative photographs of mice following 4 months of air or CS exposure and of CS-exposed mice treated with ASC during the last 2 months of exposure. Note the smaller size (girth) of CS-exposed mice and the similar size of ASC-treated CS-exposed mice compared to control mice.

Figure E1

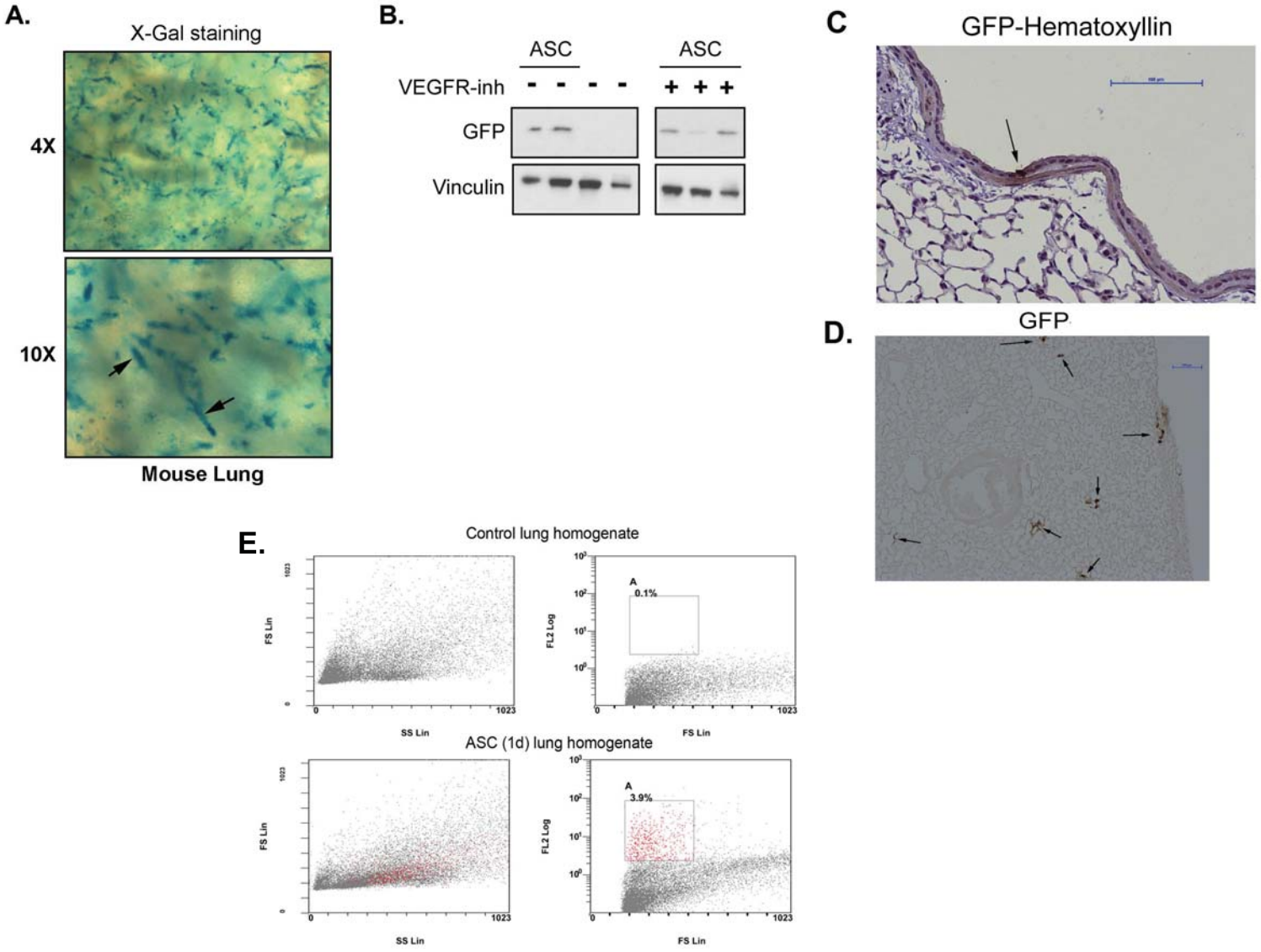
A.
X-Gal staining
4X
10X
Mouse Lung
B.
ASC
ASC
VEGFR-inh
- - - -
+ + +
GFP
Vinculin
C
GFP-Hematoxyllin
D.
GFP
E.
Control lung homogenate
FS Lin
SS Lin
FL2 Log
FS Lin
A
0.1%
ASC (1d) lung homogenate
FS Lin
SS Lin
FL2 Log
FS Lin
A
3.9%

Figure E2

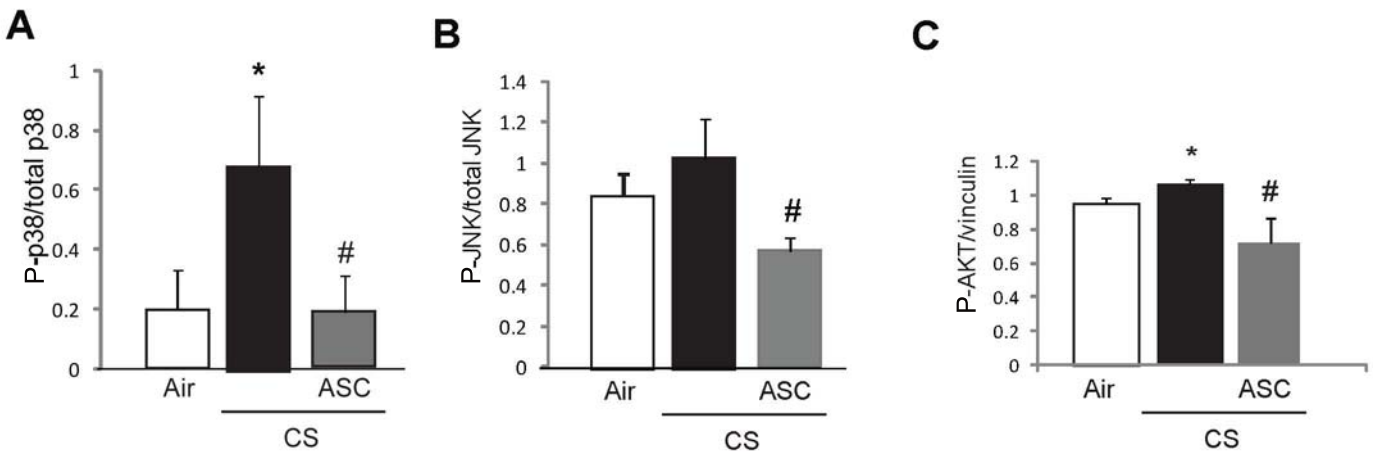
A
P-p38/total p38
1
0.8
0.6
0.4
0.2
0
*
#
Air
ASC
CS
B
P-JNK/total JNK
1.4
1.2
1
0.8
0.6
0.4
0.2
0
#
Air
ASC
CS
C
P-AKT/vinculin
1.2
1
0.8
0.6
0.4
0.2
0
*
#
Air
ASC
CS

Figure E3