**Supplemental File**

**ADAM17 protects against elastase-induced emphysema**

**by suppressing CD62L+ leukocyte infiltration in mice**

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**Supplemental Materials and Methods**

**Immunofluorescence**

Immunofluorescence experiments were performed as previously described ([1](#_ENREF_1), [3](#_ENREF_3)). In brief, lung tissue was fixed with 4% paraformaldehyde. The lung sections were deparaffinized in xylene and rehydrated in a graded ethanol series. The antigen retrieval was performed by heating sodium citrate buffer at 95°C for 20 min. Non-serum protein block (Dako, Carpinteria, CA) was applied for 30 min. The primary antibodies used were anti-MMP-12 (PA5-13181; Thermo Fisher Scientific, Waltham, MA), anti-CD62L (MEL-14, BioLegend, San Diego, CA), DyLight 488-labeled Lycopersicon esculentum (tomato) lectin (DL-1174; Vector Labs, Burlingame, CA), and anti-SP-C (sc-7706; Santa Cruz, Dallas, TX). The secondary antibodies used except for (tomato) lectin were Alexa Fluor® 488 Donkey Anti-rabbit IgG (Thermo Fisher Scientific) or Alexa Fluor® 555 Goat Anti-rat IgG (Thermo Fisher Scientific). At least 5 tissue slices were immunostained and examined with a Zeiss AxioImager microscope (Carl Zeiss, Jena, Germany).

**Flow Cytometry**

Flow cytometry analysis was performed as previously described ([2](#_ENREF_2)). In detail, the single-cell suspensions of lungs were blocked with CD16/CD32 antibody (eBioscience, San Diego, CA) for 10 min, and incubated with following primary antibody for 20 minutes: PE-Cy7-CD45 (30-F11; BioLegend, San Diego, CA), PE-Cy7-CD3 (17A2; BioLegend), PerCP-Cy5.5-CD4 (GK1.5; BioLegend), APC-Cy7-CD8a (53–6.7; BioLegend), APC-Gr-1 (RB6-8C5; BioLegend), APC-Cy7-CD11b (M1/70; BioLegend), PerCP-Cy5.5-CD11c (N418; BioLegend), FITC-CD45R/B220 (RA3-6B2; BioLegend), PE-CD45R/B220 (RA3-6B2; BioLegend), FITC-CD62L (MEL-14; BioLegend), PE-F4/80 (BM8; BioLegend). The cells were then washed twice and subsequently stained with PE-Texas Red propidium iodide (PI; BD Biosciences, San Jose, CA). The stained cells were processed by flow cytometry on the BD FACS Aria II system (BD Biosciences) or Gallios (Beckman Coulter, Brea, CA).

**Magnetic bead separation of splenic CD45+ cells**

Splenic CD45+ cells were isolated from splenocytes harvested from *Adam17flox/flox/Mx1-Cre* (*Adam17ΔMx1*) mice with a mixed genetic background (129Sv and C57BL/6) and littermate *Adam17flox/flox/Mx1-Cre−* control mice at day 0 and 35 days after elastase treatment by positive selection, using a magnetic cell sorting system (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany).

**Culture of murine** **bone marrow-derived macrophages**

Bone marrow cells were harvested from *Adam17ΔMx1* mice and littermate control mice on day 0 and 35 days after elastase treatment by flushing the femur and tibia with RPMI 1640 medium. Recovered cells were then cultured in bone marrow cell medium (20% FCS, 20 ng/ml, GM-CSF (Pepro Tech, Rocky Hill, NJ), 2mM l-glutamine, 1% penicillin/streptomycin, and 0.25 𝜇g/mL amphotericin B in RPMI 1640). Fresh bone marrow cell medium was added on day 3. On day 6, both adherent and floating cells were used as bone marrow-derived macrophages.

**Protein extraction and immunoblotting**

Splenic CD45+ cells and BMDM were lysed in 50 μL RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA) supplemented with Halt Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktail 2 (Sigma-Aldrich, St. Louis, MO). The lysate was collected by centrifugation at 14,000 g at 4 °C for 15 min, and 100 μL ddH2O was added. For immunoblotting, equal amounts (15–30 μg) of cell lysates were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Bio-Rad, Hercules, CA). Then, the proteins were transferred onto a polyvinylidene fluoride membrane (Bio-Rad). After overnight incubation with antibodies to ADAM17 (sc-13973; Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (A5441; Sigma-Aldrich, St. Louis, MO, the membrane was immunoblotted with a horseradish peroxidase-conjugated rabbit IgG antibody and visualized with enhanced chemiluminescence detection reagents (GE Healthcare). The images were analyzed using ImageJ 1.51s software (National Institutes of Health, Bethesda, MD).



**Supplemental Figure 1 Efficient knockdown of ADAM17 in ADAM17 knockout mouse embryonic fibroblasts, as well as splenic CD45+ cells and bone marrow-derived macrophages obtained from *Adam17ΔMx1* mice.** ADAM17 levels in wild type and ADAM17 knockout (KO) mouse embryonic fibroblasts (mEF), and splenic CD45+ cells and bone marrow-derived macrophages (BMDM) obtained from control, and *Adam17ΔMx1* mice were measured by immunoblotting. The data shown are representative of two independent experiments.



**Supplemental Figure 2 Soluble IL-6R and VEGFR2 levels in the bronchoalveolar lavage fluid (BALF) of control and *Adam17ΔMx1* mice.** Levels of soluble IL-6R and VEGFR2 in the BALF on days 1, 7, or 14 after porcine pancreatic elastase (PPE) injection (n = 3–6). Data represent the mean ± SEM.



**Supplemental Figure 3 Soluble** **CD62L levels in the bronchoalveolar lavage fluid (BALF) of control and *Adam17ΔMx1* mice.** Levels of CD62L in the BALF on days 0, 7, and 14 after porcine pancreatic elastase (PPE) injection measured by ELISA (n = 5–6). Data represent the mean ± SEM.



**Supplemental Figure 4 MMP-12 levels in the bronchoalveolar lavage fluid (BALF) and the whole lungs of control and *Adam17ΔMx1* mice.** Levels of MMP-12 in the BALF and the whole lungs on day 14 after porcine pancreatic elastase (PPE) injection, as measured by ELISA (n = 3–8). Levels of MMP-12 mRNA in the whole lungs on day 14, as measured by qPCR (n = 4–8). Data represent the mean ± SEM.

**Supplemental Table 1** Primer sequences used for quantitative real-time PCR

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| **Gene** | **Forward (5'-3')** | **Reverse (5'-3')** |
| *Gapdh* | TTGATGGCAACAATCTCCAC | CGTCCCGTAGACAAAATGGT |
| *Keap1* | GGCAGTGTGACAGGTTGAAG | GATCGGCTGCACTGAACTG |
| *Gpx2* | TGACCCGTTCTCCCTCATG | GCGCACGGGACTCCATAT |
| *Nqo1* | TTTAGGGTCGTCTTGGCAAC | GTCTTCTCTGAATGGGCCAG |
| *Nrf2* | TCTATGTCTTGCCTCCAAAGG | CTCAGCATGATGGACTTGGA |
| *Gclm* | TTGGGAACTCCATTCATTCA | CGGGAACCTGCTCAACTG |
| *Gclc* | TTCATGATCGAAGGACACCA | CTGCACATCTACCACGCAGT |
| *Gsr* | ATCGTGCATGAATTCCGAGT | GGTGGTGGAGAGTCACAAGC |
| *Hmox1* | CCTTCAAGGCCTCAGACAAA | GAGCCTGAATCGAGCAGAAC |
| *Mmp12* | TTTGGATTATTGGAATGCTGC | ATGAGGCAGAAACGTGGACT |

**Supplemental References**

1. **Asakura T, Ishii M, Namkoong H, Suzuki S, Kagawa S, Yagi K, Komiya T, Hashimoto T, Okamori S, Kamata H, Tasaka S, Kihara A, Hegab AE, Hasegawa N, and Betsuyaku T.** Sphingosine 1-phosphate receptor modulator ONO-4641 stimulates CD11b(+)Gr-1(+) cell expansion and inhibits lymphocyte infiltration in the lungs to ameliorate murine pulmonary emphysema. *Mucosal Immunol*, 2018.

2. **Namkoong H, Ishii M, Fujii H, Yagi K, Asami T, Asakura T, Suzuki S, Hegab AE, Kamata H, Tasaka S, Atarashi K, Nakamoto N, Iwata S, Honda K, Kanai T, Hasegawa N, Koyasu S, and Betsuyaku T.** Clarithromycin expands CD11b+Gr-1+ cells via the STAT3/Bv8 axis to ameliorate lethal endotoxic shock and post-influenza bacterial pneumonia. *PLoS Pathog* 14: e1006955, 2018.

3. **Takahashi S, Ishii M, Namkoong H, Hegab AE, Asami T, Yagi K, Sasaki M, Haraguchi M, Sato M, Kameyama N, Asakura T, Suzuki S, Tasaka S, Iwata S, Hasegawa N, and Betsuyaku T.** Pneumococcal Infection Aggravates Elastase-Induced Emphysema via Matrix Metalloproteinase 12 Overexpression. *J Infect Dis* 213: 1018-1030, 2016.