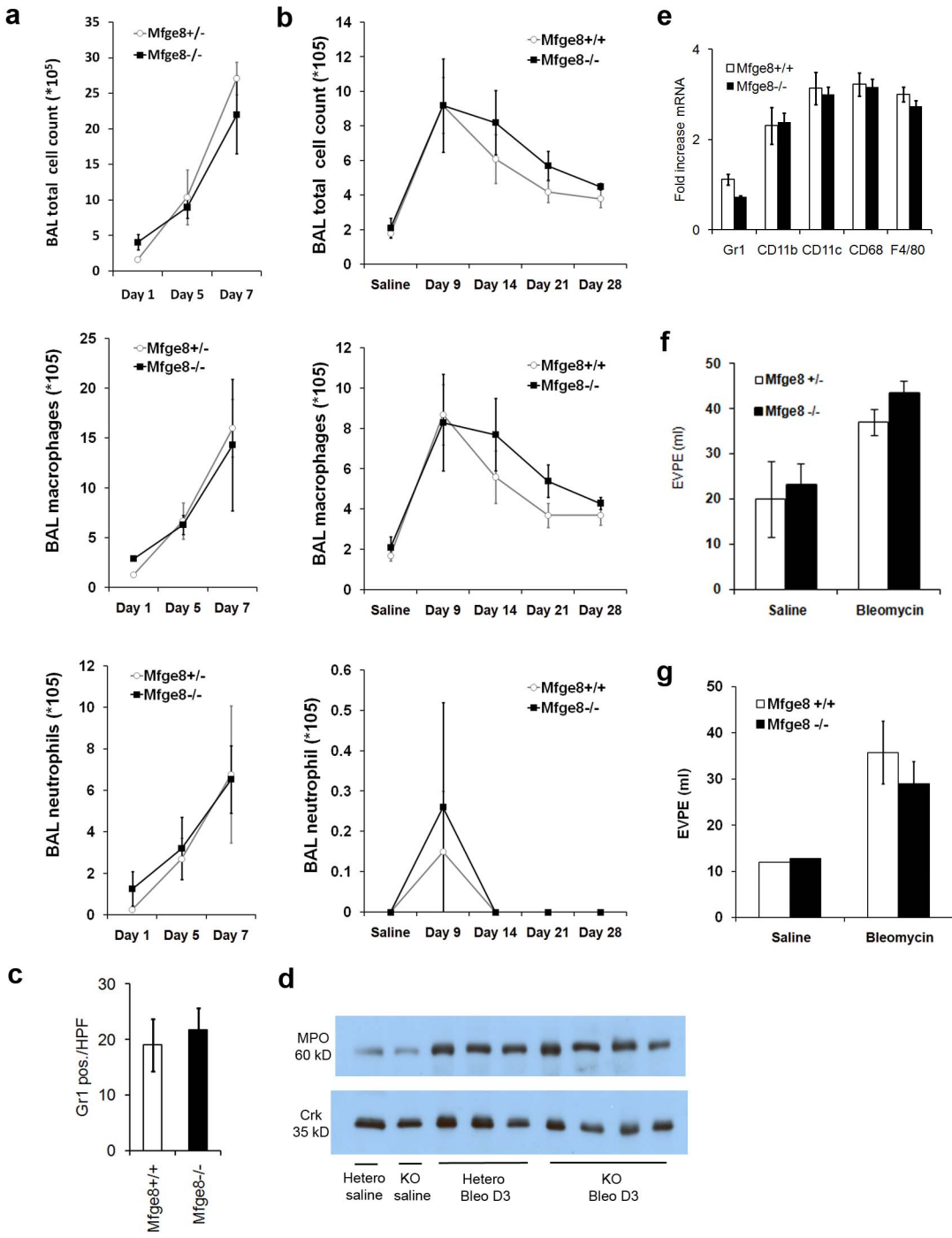
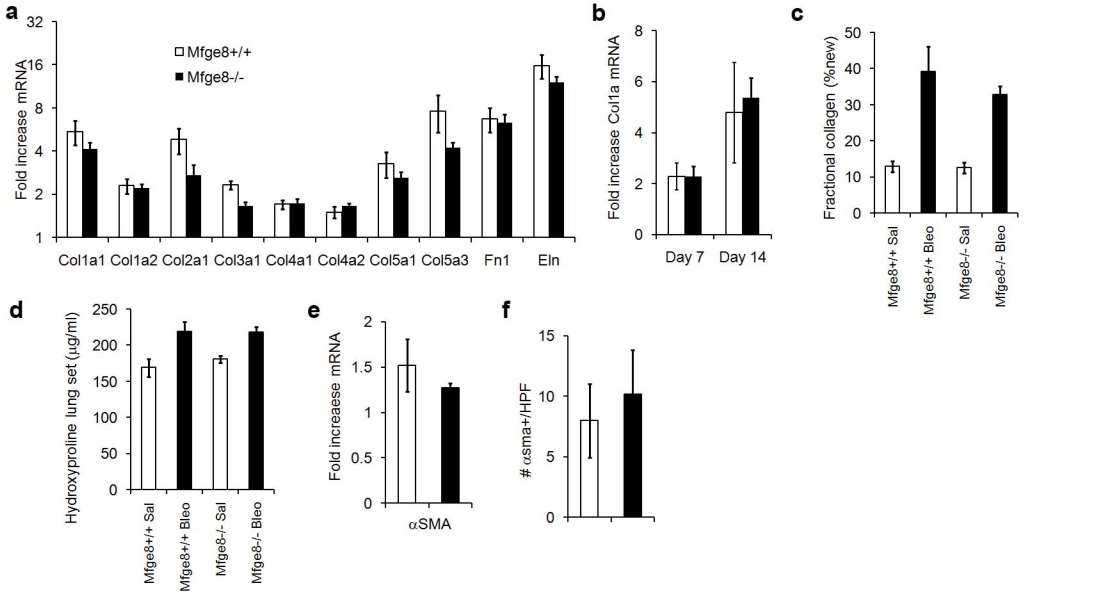
Supplementary Figure 1



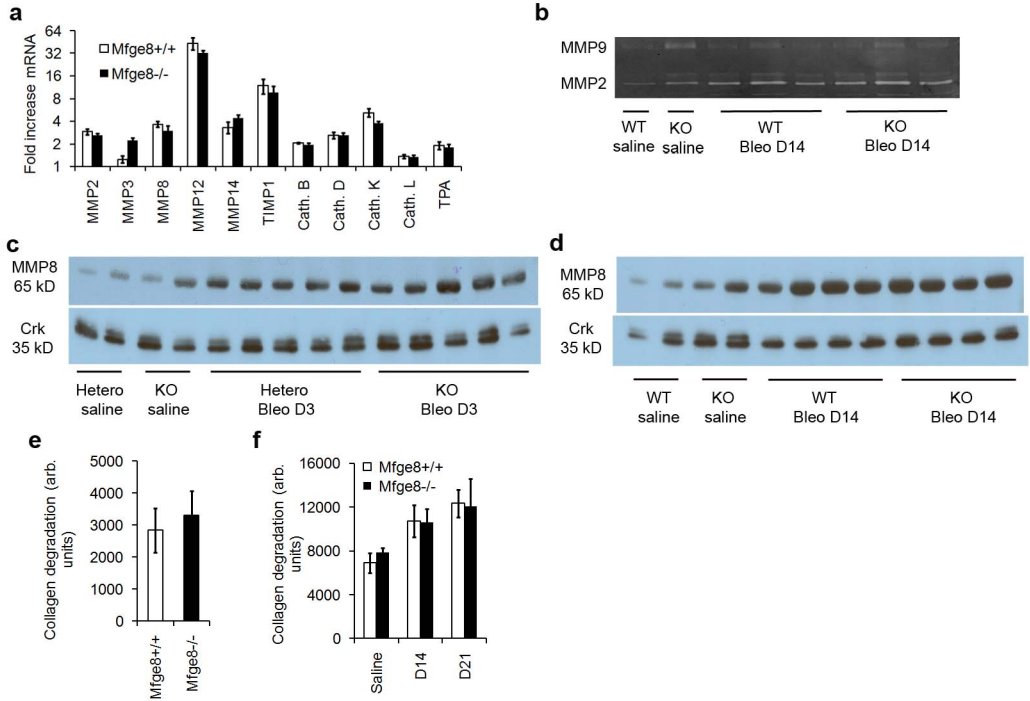
Supplemental Figure 1 Mfge8 deficiency does not affect the severity of inflammation or acute lung injury after bleomycin treatment. (a) *Mfge8-/-* and *Mfge8+/-* mice were treated with intratracheal bleomycin (5 U/kg) and inflammation was assessed by bronchoalveolar lavage counts. There was no significant difference in total cell count or macrophage or neutrophil counts between experimental groups (n=4-7). (b) *Mfge8-/-* and *Mfge8+/+* mice were treated with intratracheal bleomycin (1.1 U/kg) and inflammation was assessed by bronchoalveolar lavage counts at indicated time points. There was no significant difference in total cell count or macrophage or neutrophil counts between experimental groups, (n=3-7). (c) Tissue staining 24 hours after high-dose bleomycin (5 u/kg) for Gr1 and quantification of the number of positive cells per high-power field (200x) showed no differences between genotypes (n=3-4) (d) Myeloperoxidase (MPO) heavy chain protein levels were measured by Western blot from 7.5 μg of total lung homogenates obtained 3 days after bleomycin (5 U/kg). An antibody against Crk to ensure equal loading of protein. (e) Expression of selected markers of neutrophils and macrophages 14 days after bleomycin treatment were evaluated by expression array and quantified as fold induction relative to saline treated controls. There were no significant differences between experimental groups. (f,g) The severity of acute lung injury 5 days after bleomycin treatment (5 U/kg) was assessed by measuring extravasation of radiolabeled albumin into the lung. (f) There was no difference in vascular permeability as measured by the extravascular plasma equivalents (EPVE) between *Mfge8-/-* or *Mfge8+/-* mice (n=3 for saline treated, n=5-6 for bleomycin treated) or when comparing (g) *Mfge8-/-* with *Mfge8+/+* mice (n=1 for saline treated, n=4-5 for bleomycin treated). Data are presented as mean ± s.e.m.

Supplementary Figure 2



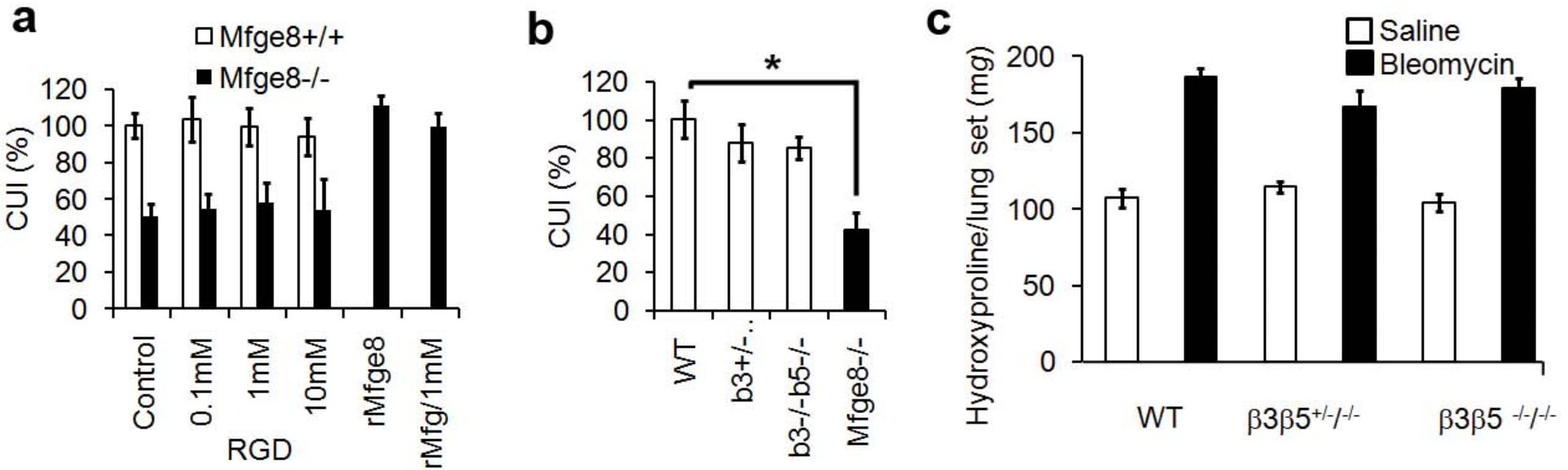
Supplementary Figure 2 Collagen production is unaffected by Mfge8 deficiency. (a) Procollagen (Col), fibronectin (Fn1), and elastin (Eln) mRNA transcripts measured by gene expression array analysis showed equal induction of transcripts taken from total lung RNA 14 days after bleomycin treatment (1.1 U/kg, n=6 pairs). (b) Procollagen 1α mRNA transcripts measured by Real-Time PCR on day 7 and 14 after bleomycin treatment (1.1 U/kg) showed no difference between experimental groups (n=6 pairs). (c) Fractional synthesis of collagen was measured 14 days after bleomycin administration (1.1 U/kg) and following 14 days of continuous labeling with deuterated water. (n=5-6 for saline and n=10-11 for bleomycin treated groups). P = 0.37 using a Students t-test to compare *Mfge8*-/- and *Mfge8*+/+ bleomycin treated mice. (d) Total lung hydroxyproline content 14 days after bleomycin administration (1.1 U/kg) is similar when comparing *Mfge8*-/- and *Mfge8*+/+ mice (n=5-6 for saline and n=10-11 for bleomycin treated groups). (e) Expression of α-smooth muscle actin mRNA transcripts by gene expression array was no different when comparing *Mfge8*-/- (■) and *Mfge8*+/+(□) 14 days after bleomycin treatment (1.1 U/kg, n=6 pairs). (f) The number of myofibroblasts per 200X field (25 randomly selected high-power fields counted per sample) in tissue sections taken 28 days after bleomycin treatment (1.1 U/kg) was quantified after staining for α-smooth muscle actin. There was no difference between *Mfge8*-/- and *Mfge8*+/+ mice. Data are presented as mean +/- s.e.m.

Supplementary Figure 3



Supplementary Figure 3 Mfge8 deficiency does not alter production or activity of proteolytic enzymes. (a) The mRNA transcript levels of metalloproteinases (MMP) and cathepsins with established roles in lung injury, TIMP1, and tissue plasminogen activator (TPA) were similar in total lung RNA taken from *Mfge8-/-* and *Mfge8+/+* mice14 days after bleomycin treatment (n=6 pairs). (b) Gelatin zymography of total lung homogenates taken from saline treated samples or samples obtained 14 days after bleomycin treatment revealed no consistent differences in MMP9 or MMP2 activity. (c) MMP8 protein levels were measured from 10 μg of total lung homogenates taken 3 days after high-dose bleomycin treatment (5u/kg) or saline treatment. An antibody against Crk to ensure equal loading of protein. (d) MMP8 protein levels measured from 10 μg of total lung homogenates taken 14 days after treatment with low-dose bleomycin(1.1u/kg) or saline. An antibody against Crk to ensure equal loading of protein. (e) Alveolar macrophages were cultured with FITC-type I collagen and collagen degradation was quantified by measuring the fluorescent signal. There were no significant differences between *Mfge8+/+* and *Mfge8-/-* macrophages (n=3 pairs). (f) 10 μg of total lung homogenates from saline treated (7 or 9 days after treatment) or bleomycin treated (days 14 and 21) were incubated with FITC-type I collagen for 60 minutes at 37°C and collagen degradation was quantified by measuring the fluorescent signal (n=3-4).Data are presented as mean +/- s.e.m.

Supplementary Figure 4



Supplemental Figure 4 RGD binding integrins do not mediate collagen uptake through Mfge8. (a) Collagen uptake by *Mfge8+/+* or *Mfge8-/-* alveolar macrophages is not inhibited by RGD peptide (n=4 pairs except for rMfg/RGD n=3) or RGE control peptide (data not shown). rMfge8 (13 μg/ml) rescue of *Mfge8-/-* collagen uptake is not inhibited by RGD peptide. (b) Alveolar macrophages taken from mice deficient in the integrin β*5* gene (β*5-/-*β*3+/-*) or β*3* and β*5* gene (β*5-/-*β*3-/-*) in the 129SVEV mouse strain have no significant impairment in collagen uptake while *Mfge8-/-* alveolar macrophages in the 129SVEV strain have a significant impairment in collagen uptake (*P = 0.001 using a Student's t-test, n=4-5). (c) Integrin β*5-/-*β*3+/-*, β*5-/-*β*3-/-* and WT mice were challenged with intratracheal bleomycin (1.1 U/kg) and total lung hydroxyproline content measured 56 days after treatment. There were no significant differences in the severity of fibrosis between experimental groups (n=6-12 for saline treated mice and n=9-12 for bleomycin treated mice). Data are expressed as mean + s.e.m.