Supplemental Data

Epithelial-Macrophage Interactions Determine Pulmonary Fibrosis Susceptibility in Hermansky-Pudlak Syndrome

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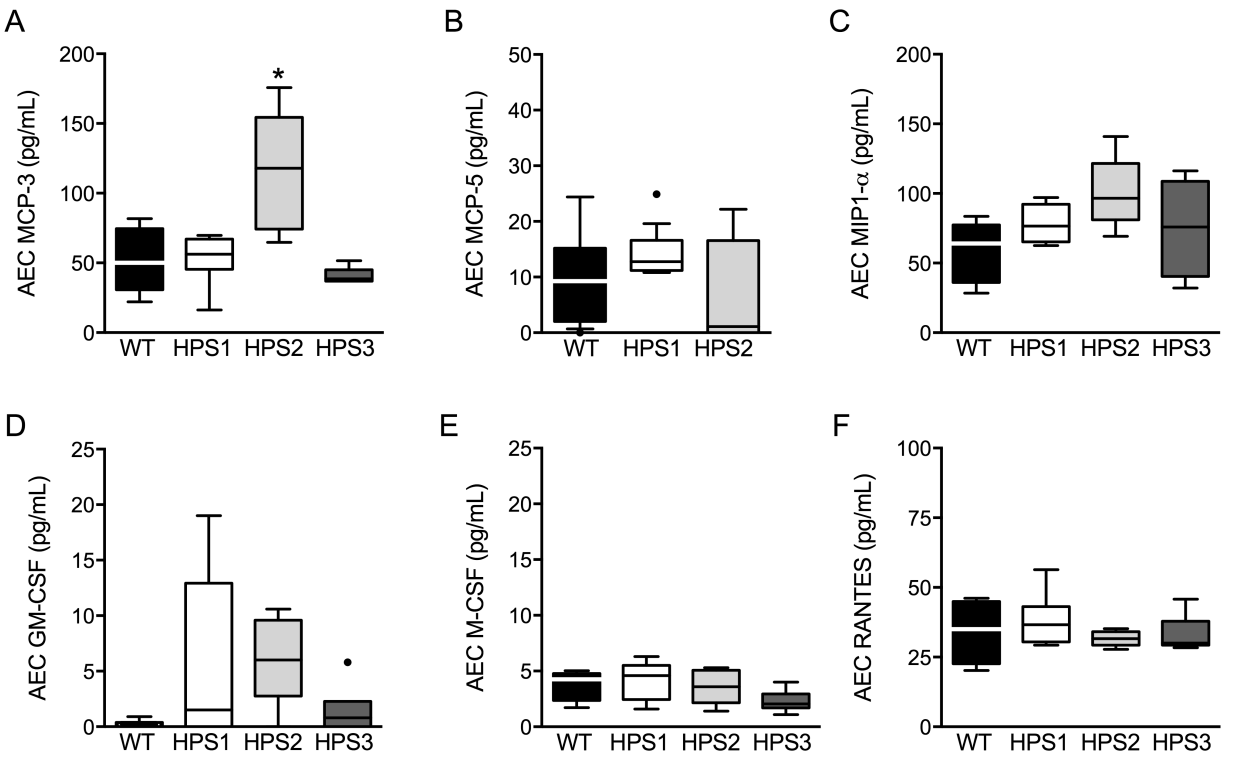
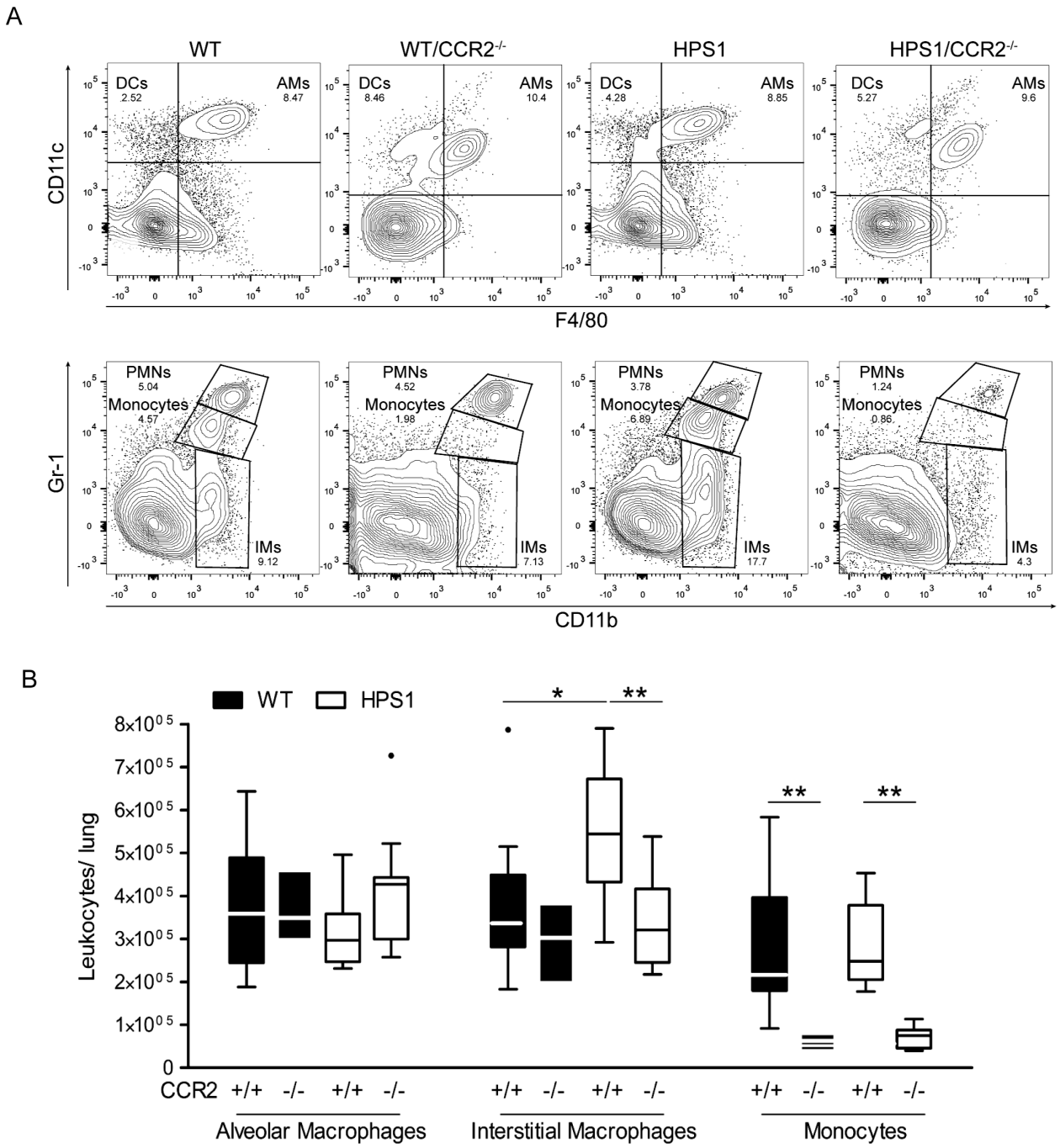
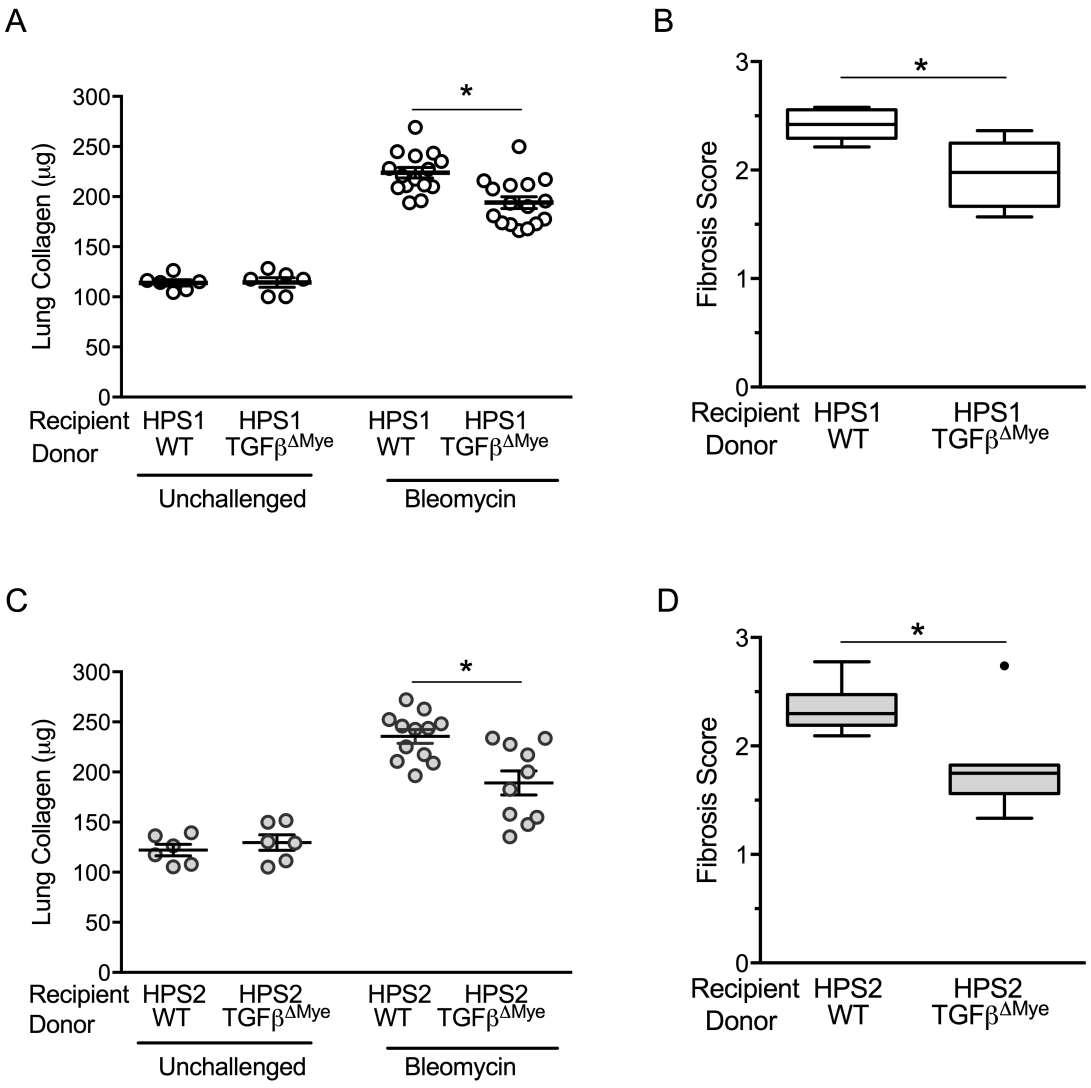


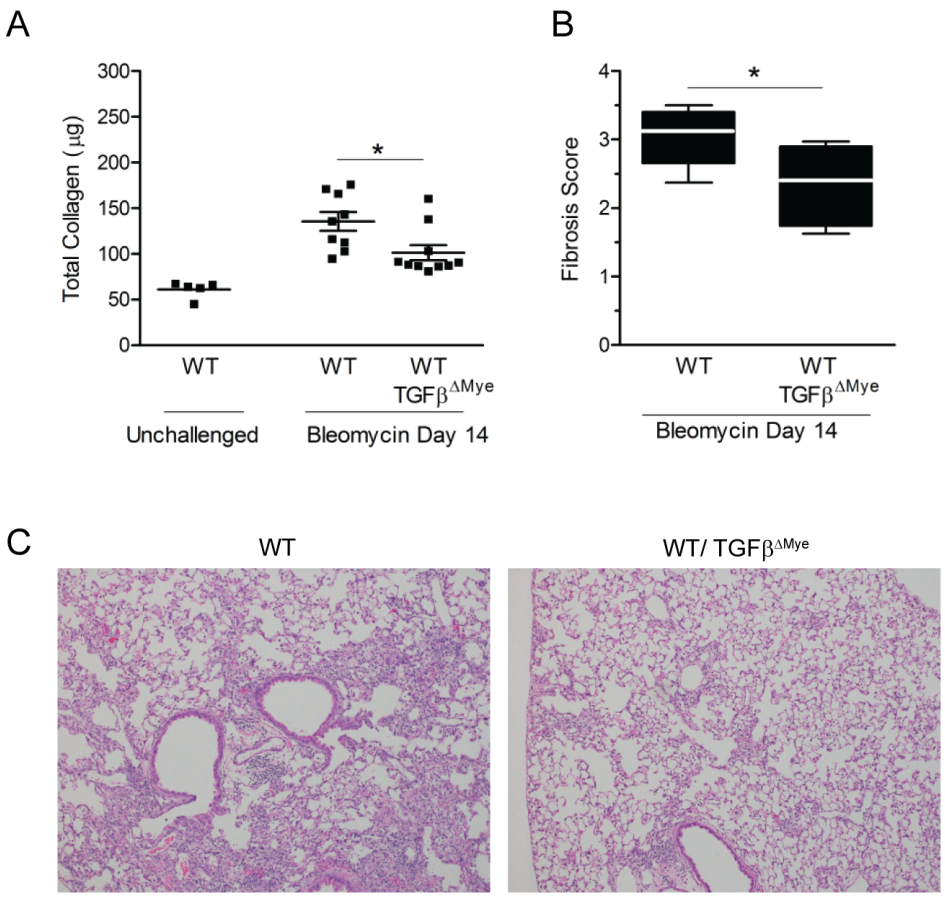
Supplemental Figure 1. Additional cytokine profile from unchallenged wildtype and HPS2 type II alveolar epithelial cells. Cytokines were measured by ELISA in cell culture media from type II alveolar epithelial cells (AECs) from unchallenged mice after 24h in culture. Data presented are box and whiskers Tukey plots. **(A)** MCP-3; n=5 WT and HPS3, n=6 HPS1 and HPS2. **(B)** MCP-5; n=9 WT, n=10 HPS1, n=6 HPS2. **(C)** MIP1α; n=8 WT and HPS2, n=5 HPS1, and n=4 HPS3. **(D)** GM-CSF; n=6 per group except n=5 HPS2. **(E)** M-CSF; n=5 WT and HPS1, n=6 HPS2 and HPS3. **(F)** RANTES; n=6 for all groups except n=5 HPS2. Comparisons between groups were conducted by Kruskal-Wallis Test with Dunn's multiple comparisons post-test; *p<0.05.



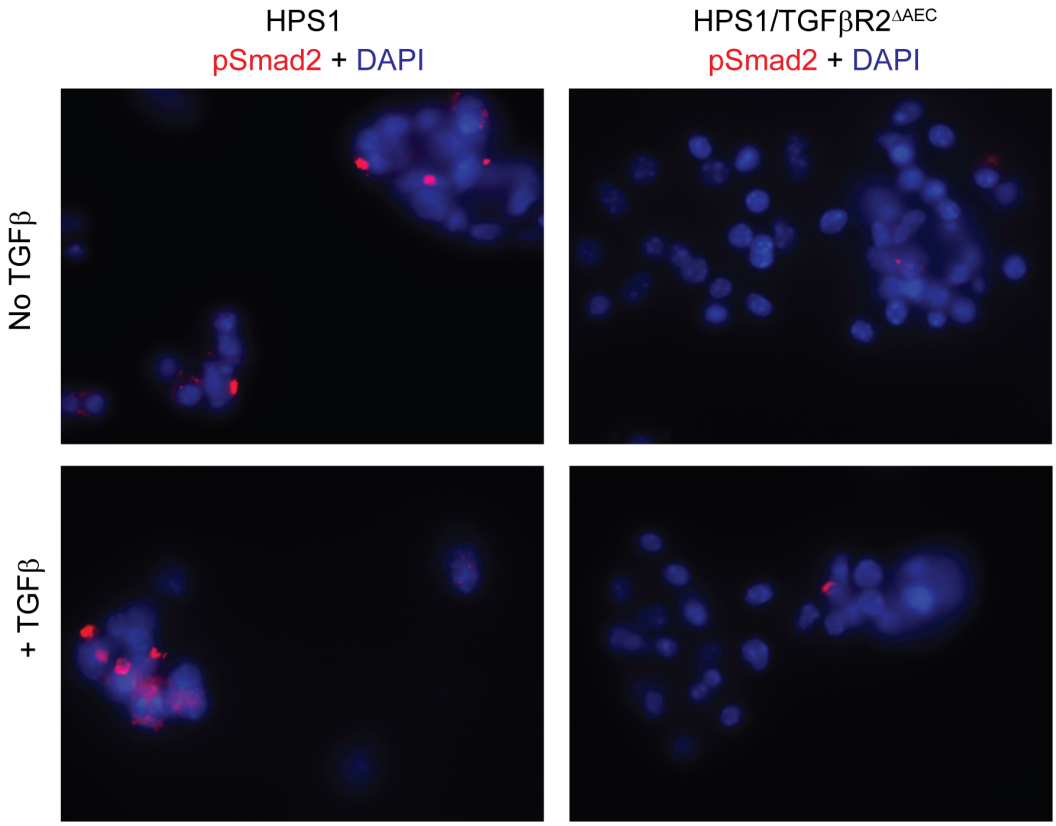
Supplemental Figure 2. HPS mice have increased numbers of CD11c^{lo}, CD11b+ interstitial macrophages, which are regulated by CCR2. Flow cytometry was used to quantitate cell populations from the lungs of unchallenged mice using the following cell surface markers: Alveolar macrophages: F4/80+, CD11c+, CD11b, Gr1 negative; Interstitial macrophages: F4/80^{lo}, CD11c^{lo}, CD11b+, Gr1 negative; Monocytes: F4/80^{lo}, CD11c negative, CD11b+, Gr1^{lo}. **(A)** Representative flow plots after CD45+ viable cell gating. **(B)** Quantitation of myeloid cell populations in WT and HPS1 mice, and with CCR2 deficiency. Data presented are box and whiskers tukey plots for n=10 for WT, n=6 WT/CCR2$^{-/-}$, n=14 for HPS1, and n=11 for HPS1/ CCR2$^{-/-}$. Comparisons between groups were conducted by Kruskal-Wallis Test with Dunn's multiple comparisons post-test; *p<0.01, **p<0.005 vs CCR2$^{-/-}$groups.



Supplemental Figure 3. Bone marrow transplant experiments demonstrate that myeloid TGFβ contributes to fibrotic susceptibility in both the HPS1 and HPS2 mouse models. **(A-D)** HPS1 and HPS2 mice underwent irradiation and transplantation with whole marrow from either WT/LysM.Cre^{+}/TGFb1$^{f/f}$ mice (denoted TGFβ$^{\Delta Mye}$) or LysM.Cre^{neg} littermate control WT mice (denoted WT). After 60 days, mice were challenged with a single dose of IT bleomycin, and the fibrotic response was determined 7 days later. **(A-B)** HPS1 recipients, n=6 per group for transplanted but non-bleomycin challenged mice. For bleomycin challenged mice, n=11 for HPS1 recipients of WT marrow and n=17 for HPS1 recipients of TGFβ$^{\Delta Mye}$ marrow. Comparisons between bleomycin challenged groups were made by Mann-Whitney *U* analysis. **(A)** Lung collagen content of left lung quantitated by Sircol assay. The values are mean ± SEM, *p<0.01. **(B)** Fibrosis Score from trichrome stained lung sections. Data presented are box and whiskers Tukey plots. *p<0.01. **(C-D)** HPS2 recipients, n=6 per group for transplanted but non-bleomycin challenged mice and n=11 per group for bleomycin challenged groups. Comparisons between bleomycin challenged HPS groups were made by Mann-Whitney *U* analysis. **(C)** Lung collagen content, *p<0.01. **(D)** Fibrosis Score, *p<0.05.



Supplemental Figure 4. Myeloid TGFβ contributes to fibrosis in wildtype mice.
The LysM.Cre promoter was used to generate mice deficient in myeloid TGFβ by crossing LysM.Cre+ mice with TGFb1 floxed mice (LysM.Cre^{+}/TGFb1$^{f/f}$ mice; denoted WT/TGFβ$^{\Delta Mye}$) and studied in comparison to LysM.Cre^{neg} littermate control mice (LysM.Cre^{neg}/TGFb1$^{f/f}$(denoted WT)). **(A)** Lung collagen content of left lung was quantitated by the Sircol assay at 14 days after IT bleomycin (0.05 units). The values are mean ± SEM. Comparisons between bleomycin challenged groups were made by Mann-Whitney *U* analysis with n=9 for WT and n=10 for WT/TGFβ$^{\Delta Mye}$, **p*<0.01. **(B)** Fibrosis Scores from Trichrome stained lung sections of the right lung, **p*<0.01. Data presented are box and whiskers Tukey plots, **p*<0.05. **(C)** Lung histology at 14 days after IT bleomycin. Representative H&E images (10x original magnification) are shown from a WT littermate control mouse (denoted WT) and from a WT/TGFβ$^{\Delta Mye}$ mouse.



Supplemental Figure 5. Reduction in TGFβ signaling in alveolar epithelial cells isolated from HPS1 mice with epithelial deletion of TGFBR2. Type II alveolar epithelial cells (AECs) were isolated from unchallenged HPS1/SPC.Cre^{+}/TGFBR2$^{f/f}$ (denoted HPS1/TGFBR2$^{\Delta AEC}$) or HPS1/SPC.Cre^{neg} /TGFBR2$^{f/f}$ littermate control mice (HPS1). Cells were stimulated with recombinant TGFβ (10 ng/ml) for 2 hours or vehicle control, then fixed in 4% paraformaldehyde and stained with rabbit polyclonal psmad2 primary antibody followed by Cy3 conjugated secondary antibody. Nuclear staining was done with DAPI using Vectashield mounting medium. Confocal fluorescent images were captured using an Olympus IX81 Inverted research microscope configured with an Olympus IX2 biological disk-scanning unit (Olympus, Tokyo, Japan). Representative images (400X) from 2 separate experiments are shown.

Supplemental Table 1. Mouse Models

Mouse Model Abbreviation	Murine Strain and Mutation	Human Disease Correlate	Protein	Rationale
Wildtype (WT)	C57BL/6J	n/a	n/a	Wildtype controls
HPS1	Homozygous HPS1 mutations; naturally occurring ('pale ear' mouse	HPS-1	Novel, BLOC3	Correlate for HPS human subtype most commonly associated with pulmonary fibrosis
HPS2	Homozygous mutations in *AP3b1*; naturally occurring in ('pearl' mouse)	HPS-2	Adaptor protein AP-3	Correlate for HPS human subtype associated with interstitial lung disease; AP-3 amenable to study
HPS2/TG+	HPS2mt with transgenic correction of AP3b1 in the lung epithelium	n/a	Adaptor protein AP-3	Transgenic model previously developed
HPS3	Homozygous HPS3 mutations; naturally occurring ('cocoa' mouse)	HPS-3	Novel, BLOC2	Correlate for HPS human subtype not associated with pulmonary fibrosis
$CCR2^{-/-}$	Global knockout of CCR2 (homozygous)	n/a	CCR2	Receptor for MCP-1; eliminates MCP-1 signaling
$MCP1^{\Delta AEC}$	Lung epithelial specific deletion of MCP-1 using the SPC.Cre promoter	n/a	MCP-1	Model developed for deletion of MCP-1 in epithelial cells
$TGF\beta^{\Delta Mye}$	Myeloid specific deletion of TGFB1 using the LysM.Cre promoter	n/a	TGFβ	Model developed for deletion of TGFβ in myeloid cells
$TGFBR2^{\Delta AEC}$	Lung epithelial specific deletion of TGFBR2 using the SPC.Cre promoter	n/a	TGFBR2	Model developed for deletion of TGFBR2 and attenuation of TGFβ signaling in lung epithelial cells