

Mesenchymal Stem Cells Exert their Anti-Asthmatic Effects Through Macrophage Modulation in a Murine Chronic Asthma Model

Anti-Asthmatic Effects of Mesenchymal Stem Cells

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Supplemental Figure I.

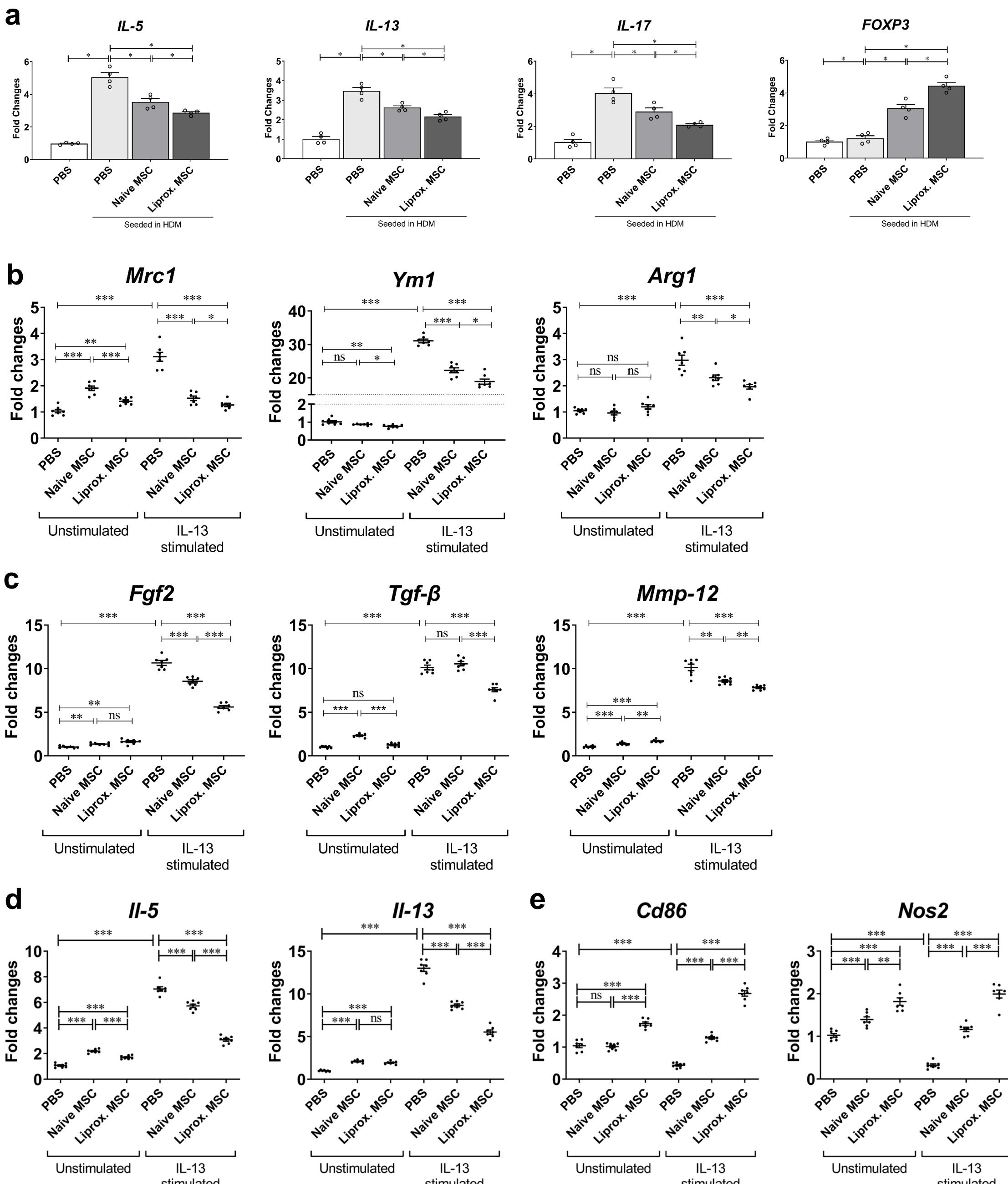


FIGURE S1. Effects of naïve and Liproxstatin-1-primed hUC-MSCs on human PBMCs or murine alveolar macrophages.

(a) Human PBMCs obtained from HDM-sensitized patients were subjected to RT-qPCR to evaluate the expression of IL-5, IL-13, IL-17, and FOXP3, the representative markers of Th2, Th17, and regulatory T cells. (b-e) Evaluation of mRNA expression of macrophages associated genes of M2 activation (b), airway remodeling (c), type 2 cytokines (d), and M1 activation (e). Each value in these panels is from a different individual and the mean \pm SEM is illustrated. All results are representative of at least three independent experiments. *p < 0.05, **p < 0.01, *** p < 0.001, ns, not significant. (by Kruskal-Wallis test using GraphPad Prism 7, <https://www.graphpad.com>)

PBMC, Peripheral blood mononuclear cell; Fgf2, fibrosis growth factor 2; Tgf-β, transforming growth factor-beta; Mmp-12, matrix metallopeptidase-12; IL, Interleukin.

Supplemental Figure II.

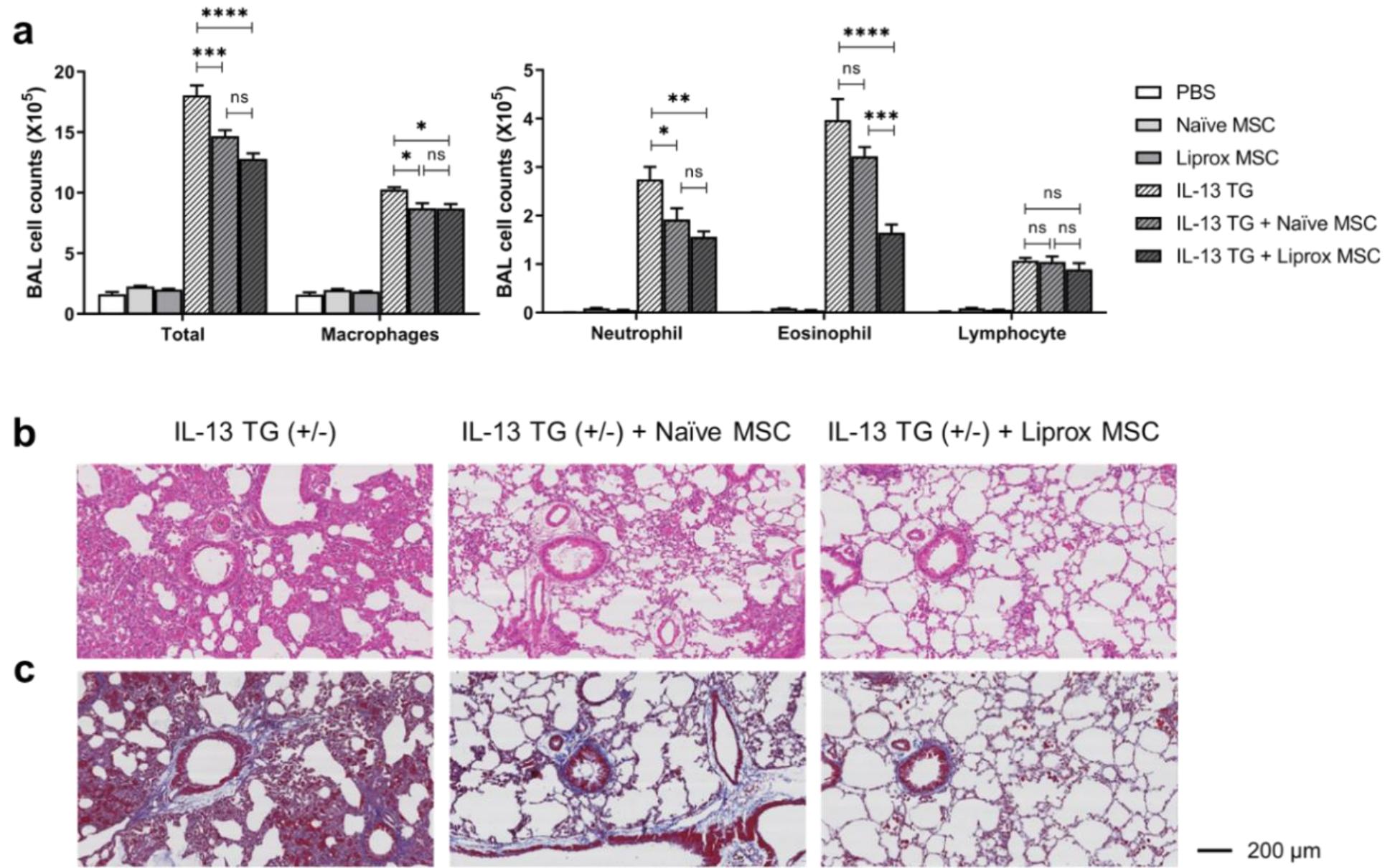


FIGURE S2 Different effects of Naïve MSC and Liproxstatin-1-primed MSC on IL-13 TG mice.

(a) shows the result of total cell counts and differential counts of macrophages, neutrophils, eosinophils, and lymphocytes in BAL fluid. (b-c) shows a histological comparison between the groups. H&E (b) and PAS (c) stains were used. One-way ANOVA and Tukey's analysis were used and error bars represent SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, ns stands for not significantly different.

Supplemental Figure III.

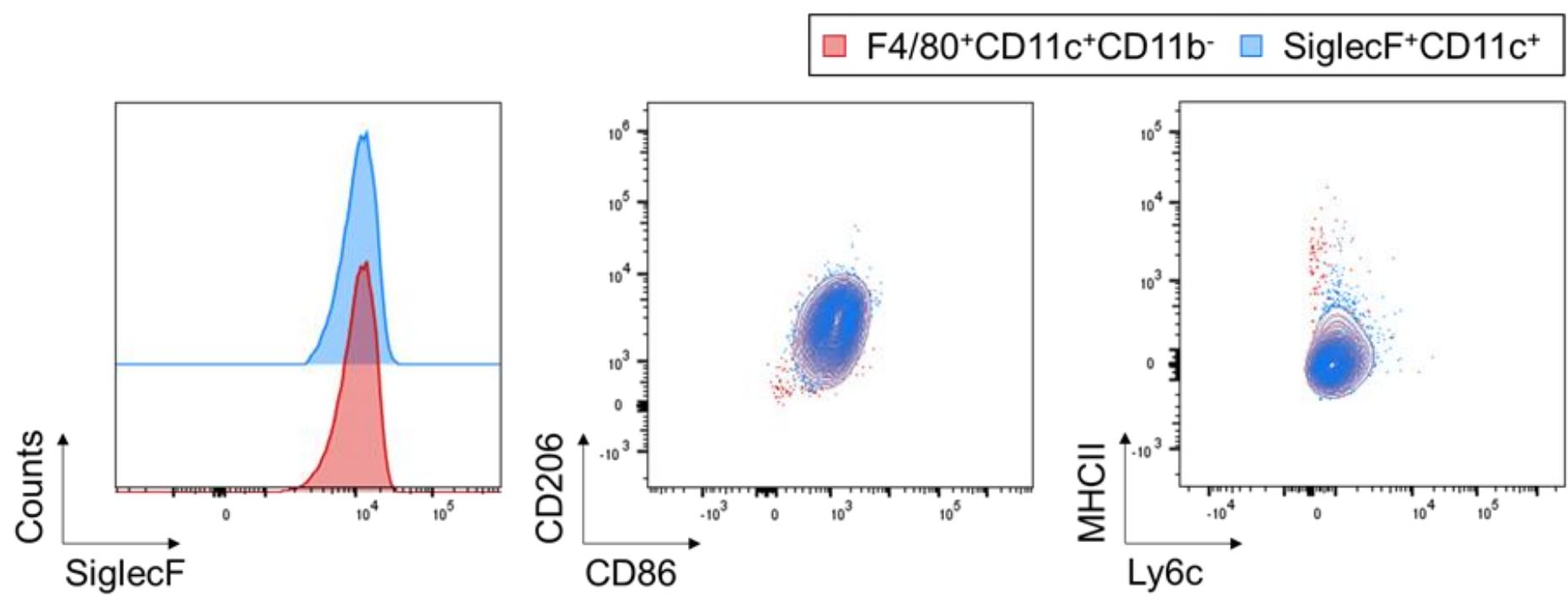


FIGURE S3 Comparison of the expression of CD206, CD86, MHCII, Ly6c, and SiglecF on SiglecF⁺CD11c⁺ and F4/80⁺CD11c⁺CD11b⁻ alveolar macrophages assessed by flow cytometry

Supplemental Figure IV.

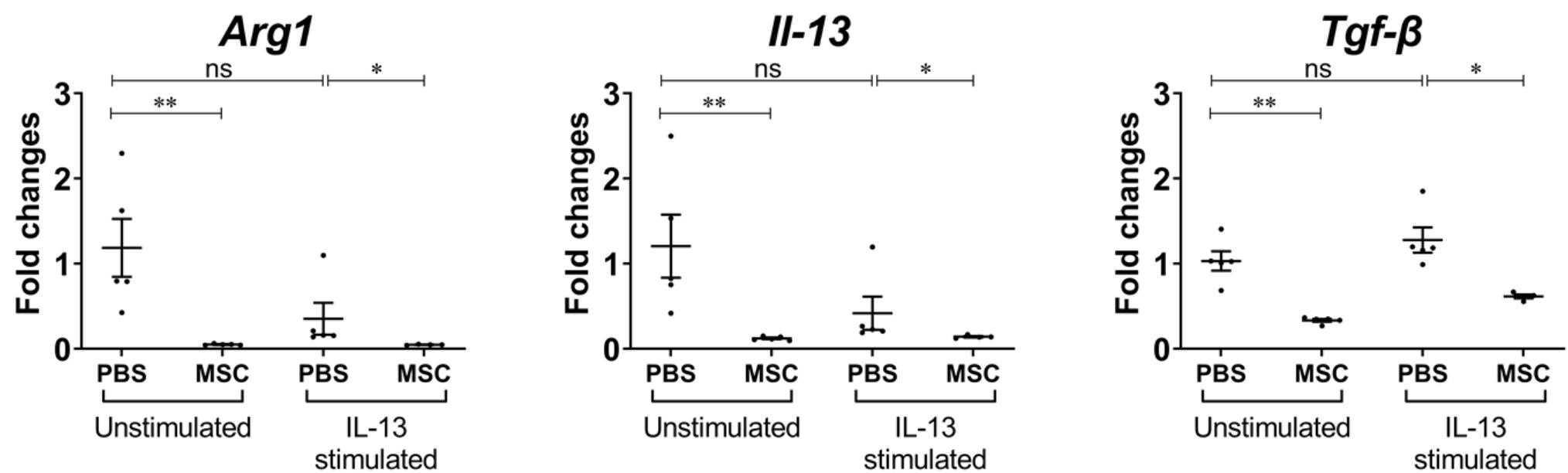


FIGURE S4 Effects of Liproxstatin-1-primed hUC-MSCs on murine macrophages.

Supplemental Table I. The primer sequences for RT-qPCR

| Gene | Forward | Reverse |
|---------------|---------------------------------------|--|
| Human | | |
| <i>IL-5</i> | 5'-AGC TGC CTA CGT GTA TGC CA-3' | 5'-GCA GTG CCA AGG TCT CTT TCA-3' |
| <i>IL-13</i> | 5'-TGA GGA GCT GGT CAA CAT CA-3' | 5'-CAG GTT GAT GCT CCA TAC CAT-3' |
| <i>IL-17</i> | 5'-GGA CTG TGA TGG TCA ACC TGA-3' | 5'-TCA TGT GGT AGT CCA CGT TCC-3' |
| <i>FOXP3</i> | 5'-TCA TCC GCT GGG CCA TCC TG-3' | 5'-GTG GAA ACC TCA CTT CTT GGT C-3' |
| <i>GAPDH</i> | 5'-TGT AGA CCA TGT AGT TGA GGT CA-3' | 5'-AGG TCG GTG TGA ACG GAT TTG-3' |
| Mouse | | |
| <i>Mrc1</i> | 5'-CAA GGA AGG TTG GCA TTT GT-3' | 5'-CCT TTC AGT CCT TTG CAA GT-3' |
| <i>Yml</i> | 5'-GGG CAT ACC TTT ATC CTG AG-3' | 5'-CCA CTG AAG TCA TCC ATG TC-3' |
| <i>Arg1</i> | 5'-CTC CAA GCC AAA GTC CTT AGA G-3' | 5'-AGG AGC TGT CAT TAG GGA CAT C-3' |
| <i>Fgfl</i> | 5'-GGG AGA TCA CAA CCT TCG CA-3' | 5'-CTG TCC CTT GTC CCA TCC AC-3' |
| <i>Fgf2</i> | 5'-GAA ACA CTC TTC TGT AAC ACA CTT-3' | 5'-GTC AAA CTA CAA CTC CAA GCA G-3' |
| <i>Fn1</i> | 5'-CAC GGG AGC CTC GAA GAG-3' | 5'-ACA ACC GGG CTT GCT TTG-3' |
| <i>Tgf-β</i> | 5'-AGG AGA CGG AAT ACA GGG CT-3' | 5'-CCA CGT AGT AGA CGA TGG GC-3' |
| <i>Mmp-9</i> | 5'-AAC CTC CAA CCT CAC GGA CA-3' | 5'-TCA TCG ATC ATG TCT CGC GG-3' |
| <i>Mmp-12</i> | 5'-TCA GTC CCT CTA TGG AGC CC-3' | 5'-CAC AGA TGC AGA GAA GCC CA-3' |
| <i>Muc5ac</i> | 5'-GGA ACT GTG GGG ACA GCT CTT-3' | 5'-GTC ACA TTC CTC AGC GAG GTC-3' |
| <i>Nos2</i> | 5'-ACA TCG ACC CGT CCA CAG TAT-3' | 5'-CAG AGG GGT AGG CTT GTC TC-3' |
| <i>Chil3</i> | 5'-AGA AGG GAG TTT CAA ACC TGG T-3' | 5'-GTC TTG CTC ATG TGT GTA AGT GA-3' |
| <i>Hprt1</i> | 5'-CTG GTG AAA AGG ACC TCT CGA AG-3' | 5'-CCA GTT TCA CTA ATG ACA CAA ACG-3' |
| <i>Gapdh</i> | 5'-GGA GTC AAC GGA TTT GGT CGT A-3' | 5'-CAA CAA TAT CCA CTT TAC CAG AGT TA-3' |