Figure Legends for Supplementary materials (Tateyama et al.)

Supplementary material, Figure S1. Sorting strategy for macrophage purification. (a) Procedure for the purification of BMDMs (associated with Fig. 1d). M-BMDMs were sorted as $F4/80^+$ $CD11b^+$ cells. R1, sorted region. A representative result of 3 independent experiments is shown. (b) Procedure for the purification of thioglycollate-elicited macrophages (associated with Fig. 1f). Five days after the thioglycollate injection, macrophages were purified from peritoneal cavity cells as $F4/80^+$ $CD11b^+$ cells after excluding Siglec-F^{high} cells. R1, sorted macrophage fraction. Siglec-F^{high} cells were sorted as eosinophils for comparison. A representative result of 4 independent experiments is shown.

Supplementary material, Figure S2. GM-CSF induces Siglec-F expression on M-BMDMs. Cells were stimulated for 24 h and cell surface expression of Siglec-F was examined by indirect staining. Anti-Siglec-F antibody (R&D Systems) and control rat IgG (Santa Cruz Biotechnology) were used for primary antibody. A representative result of 3 independent experiments is shown.

Supplementary material, Figure S3. IL-3 enhances Siglec-F expression in BMDMs. M-BMDMs were stimulated with IL-3 for 24 h and Siglec-F expression was examined by qRT-PCR. Data are the mean ± SE of 5 independent experiments. *, $p < 0.05$ versus none by the Student's *t*-test.

Supplementary material, Figure S4. Siglec-F knockdown reduces STAT6 phosphorylation induced by IL-4 in M-BMDMs. (a) Schematic presentation of the knockdown experiment. M-BMDMs were transfected with Siglec-F or control siRNA. Cells were washed after a 48-h culture, and stimulated with IL-4 for 60 min. The phosphorylation of STAT6 was examined by Western blotting. (b) Confirmation of knockdown. Total RNA was collected 24 h after the stimulation and subjected to qRT-PCR. Data are the mean ± SE of 3 independent experiments. *, $p < 0.05$ versus the control by the Student's *t*-test. (c) Siglec-F knockdown reduced the phosphorylation of STAT6. Total and the phosphorylated form of STAT6 were examined. Actin was measured as a control. A representative result of 7 independent experiments is shown. (d) Quantification of band intensity. The band intensity of pSTAT6 was normalized to that of actin. The band intensity of control siRNA was regarded as 1. Data are the mean ± SE of 7 independent experiments. *, $p < 0.05$ versus the control by the Student's *t*-test.

Supplementary material, Figure S5. Siglec-F knockdown reduces STAT6 phosphorylation induced by IL-4 in L929-BMDMs. (a) Schematic presentation of the knockdown experiment. L929-BMDMs were transfected with Siglec-F or control siRNA. Cells were washed after a 48-h culture, and stimulated with IL-4 for the indicated periods. The phosphorylation of STAT6 was examined by Western blotting. (b) Siglec-F knockdown reduced the phosphorylation of STAT6. Total and the phosphorylated form of STAT6 were examined. Actin was measured as a control. A representative result of 3 independent experiments is shown. (c) Quantification of band intensity. The band intensity of pSTAT6 was normalized to that of actin. The band intensity of control siRNA at 15 min was regarded as 1. White and black bars indicate control and Siglec-F

siRNAs, respectively. Data are the mean ± SE of 3 independent experiments. *, $p < 0.05$ versus the control at the same time point by the Student's *t*-test.

Supplementary material, Figure S6. Effects of Siglec-F knockdown on the stimulation of LPS plus IFN-γ in M-BMDMs. (a-c) Effects of Siglec-F knockdown on gene expression induced by LPS plus IFN-γ. (a) Schematic presentation of the knockdown experiment. M-BMDMs were transfected with Siglec-F or control siRNA in the presence of GM-CSF. Forty-eight hours later, cells were washed and stimulated with LPS plus IFN-γ for an additional 4 h. The expression of Siglec-F, iNOS, IL-10, and TNF-α was measured by qRT-PCR. (b) Confirmation of Siglec-F knockdown at 52 h. (c) Effect of Siglec-F knockdown on the gene expression. Data are the mean ± SE. N=3 (iNOS), 4 (IL-10), 3 (TNF-α). *, $p < 0.05$ versus the control by the Student's *t*-test. (d-f) The effects of Siglec-F knockdown on IκBα degradation induced by LPS plus IFN-γ. (d) Schematic presentation of the knockdown experiment. M-BMDMs were transfected with Siglec-F or control siRNA in the presence of GM-CSF. Cells were washed after a 48-h culture, and stimulated with LPS plus IFN-γ for 60 min. The amount of IκBα was assessed by Western blotting. (e) Siglec-F knockdown did not affect IκBα degradation. A representative result of 3 independent experiments is shown. (f) Quantification of band intensity. The band intensity of IκBα was normalized by that of actin. The band intensity of control siRNA without a stimulation was regarded as 1. White and black bars indicate control and Siglec-F siRNAs, respectively. Data are the mean ± SE of 3 independent experiments.

Supplementary material, Figure S7. Siglec-F knockdown enhances the STAT5

phosphorylation induced by GM-CSF in M-BMDMs. (a) Schematic presentation of the knockdown experiment. M-BMDMs were transfected with Siglec-F or control siRNA. Cells were washed after a 48-h culture, and stimulated with GM-CSF for 60 min. (b) Phosphorylation was assessed by Western blotting. A representative result of 7 independent experiments is shown. Total and the phosphorylated form of STAT5 were examined. Actin was measured as a control. (c) Quantification of band intensity. The band intensities of pSTAT5 were normalized to that of actin. The band intensity of control siRNA was regarded as 1. Data are the mean ± SE of 7 independent experiments. *, $p < 0.05$ versus the control by the Student's *t*-test.