Supporting Information

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SI Materials and Methods

Human Alpha-1-Antitrypsin and Albumin. Clinical-grade human alpha-1-antitrypsin (hAAT) (Aralast) and human albumin were obtained from Baxter. Rapamycin was purchased from LC Laboratories, reconstituted in ethanol at 10 mg/mL, and diluted in 5% Tween-80 (Sigma) and 5% PEG-400 (Hampton Research). Rapamycin was injected at 4 mg/kg, i.p. daily from 1 d before bone marrow transplantation (BMT). LPS from *Escherichia coli* O55:B5 was purchased from Sigma.

Mice. Female C57BL/6 (B6, H-2^b, CD45.2^+, CD229.1^-) C3H.SW (H-2^b, CD45.2^+, CD229.1^+) mice were purchased from the Jackson Laboratories. B6-Ly5.2 (H-2^b, CD45.1^+, CD45.2^-, CD229.1^-), B6D2F1 (B6, H-$2^{b/d}$, CD45.2^+, CD229.1^-) mice were purchased from the Charles River Laboratories. GFP-FoxP3 knock-in mice (GFP-Foxp3, H-2^b, CD45.1^+, CD45.2^+, CD229.1^-) were provided by Alexander Rudensky (University of Washington, Seattle) and then bred at the University of Michigan animal facility.

Bone Marrow Transplantation. Bone marrow transplantation was performed as described (1–4). Briefly, splenic T cells from donor mice were enriched by the MACS cell separation system using anti-CD90.2 microbeads or a pan T-cell isolation kit (Miltenyi). Bone marrow T cells were depleted using anti-CD90.2 microbeads. Recipient mice were irradiated (^{137}Cs source) with 10 Gy total-body irradiation on day −1 and injected with either syngeneic or allogeneic T cells (1–2 × 10^6 or 2 × 10^5) along with 4–5 × 10^6 T-cell–depleted bone marrow cells. For the F_1 recipient, 10 Gy can be nonlethal; however, these recipient mice develop graft-versus-host disease (GvHD) regardless of the intensity of radiation once hybrid resistance has been overcome (5, 6). Mice were housed in sterilized microisolator cages and received normal chow and autoclaved hyperchlorinated water for the first 3 wk after BMT and filtered water thereafter. Survival was monitored daily, and clinical GvHD score was assessed weekly. Animal studies were approved by the University of Michigan Committee on the Use and Care of Animals.

Bone Marrow Dendritic Cell Generation. Dendritic cells (DC) were generated as described (7). Briefly, bone marrow cells were isolated from mouse femurs and tibias and cultured in the presence of 20 ng/mL recombinant murine GM-CSF (Peprotech) for 7 d. CD11c$^+$ dendritic cells were isolated from bone marrow culture with the MACS cell separation system using CD11c microbeads (Miltenyi).

Flow Cytometric Analysis. Flow cytometric analysis was performed using FITC, phycoerythrin (PE), PerCP-Cy5.5, or antigen-presenting cell-conjugated monoclonal antibodies to mouse CD4 (clone RM4-4), CD229.1 (30C7) (BD Pharmingen), CD8a (53-6.7), CD25 (PC61.5), CD45.1 (A20), and CD45.2 (104) (eBioscience). Cells were stained and analyzed on a FACSVantage SE (Becton Dickinson) or C6 cytometer (Accuri Cytometers) as described (1–4).

Electrophoretic Mobility Shift Assay. CD11C$^+$ DCs were isolated from 16 B6 mice, uniformly divided into four dishes (100 mm), treated with human albumin (hALB) (1 mg/mL) or hAAT (1 mg/mL) for 4 h, and then treated with LPS (500 ng/mL) or diluent for another 3 h (8). Nucleic extracts were incubated with ^{32}P γ-ATP–labeled probe specific for NF-κB binding, derived from the class I MHC gene promoter for 30 min at room temperature. After reaction, the samples were separated by 5% polyacrylamide gel, dried, and visualized by autoradiography. Probe sequences used were forward (5′-AGTTGAGGGGACTTTCCCAGGC-3′) and reverse (5′-GCCTGGGAAAGTCCCCTCAACT-3′).

ELISA. ELISAs for TNF-α, IL-1β, IL-6, and IL-10 (BD Pharmingen) were performed according to the manufacturer's protocol.

In Vitro Suppression Assay. CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ T cells were isolated from spleen cells from BALB/c mice using the MACS cell separation system (Miltenyi). The purity of CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ T cells was >85%. CD4$^+$CD25$^+$ T cells were serially diluted from 2 × 10^4 to 2,500 cells/well and incubated with 2 × 10^4 CD4$^+$CD25$^-$ T cells and 500 or 2,500 allogeneic B6 bone marrow-derived dendritic cells for 72, 96, or 120 h. Incorporation of ^{3}H-thymidine (1 μCi/well) by proliferating cells was measured during the last 12 h of culture, as described (1–4).

Statistical Analysis. Survival curves were plotted and compared by log-rank analysis; $P < 0.05$ was considered statistically significant. A paired t test was used to evaluate significant differences between groups in in vitro experiments. Data are expressed as mean ± SEM.

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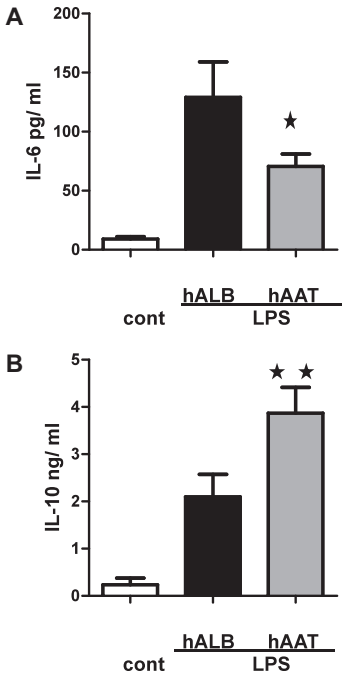

Fig. S1. Alpha-1-antitrypsin modulates cytokine secretion by macrophages. Peritoneal macrophages were obtained from C57BL/6 animals and preincubated overnight with 4 mg/mL concentration of hAAT or hALB and then stimulated for 8 h with LPS (100 ng/mL). Cytokine IL-6 and IL-10 levels in the supernatants were measured by ELISA. $*P < 0.05$.

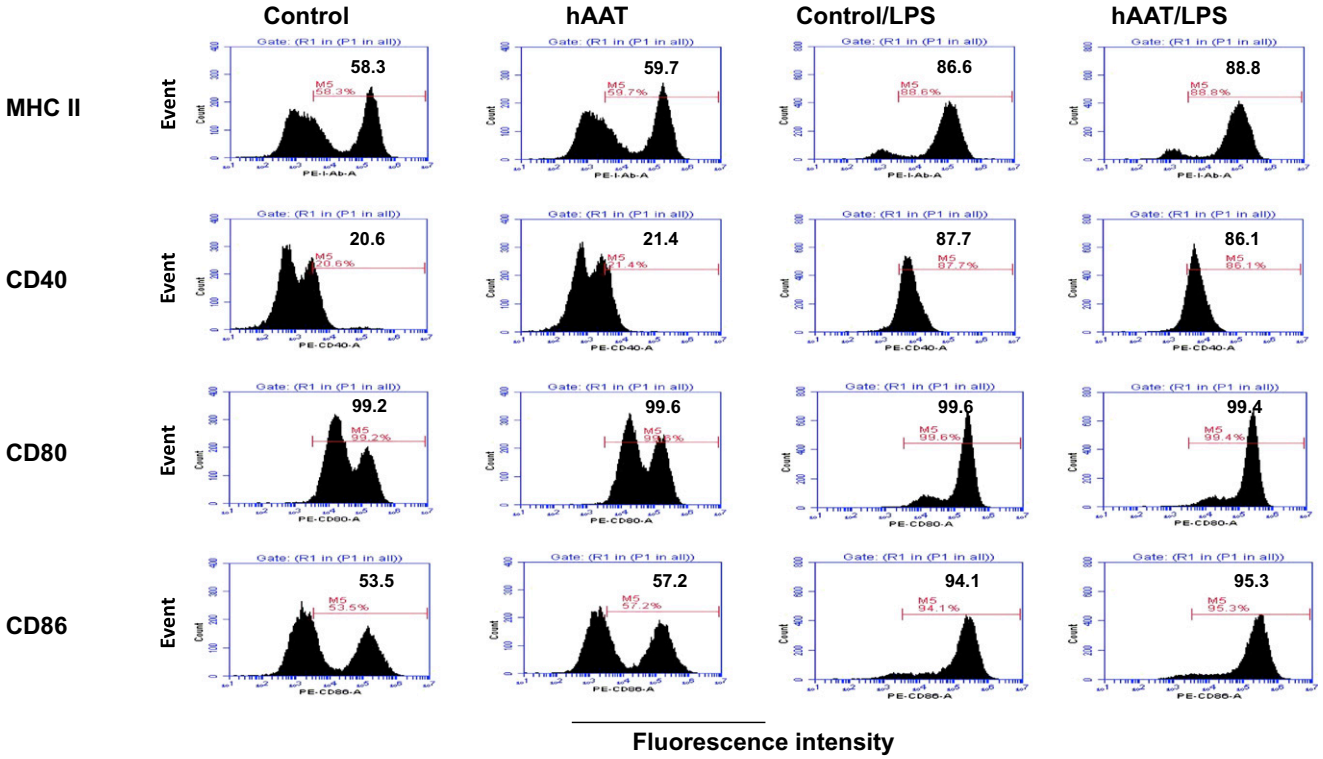

Fig. S2. Effect of hAAT on DC maturation. B6 BM cells were cultivated in the presence of GM-CSF (20 ng/mL) for 7 d. hAAT (0.5 mg/mL) was added on day 4. Day 7 BM culture cells were stimulated with LPS (500 ng/mL) for 16 h. Cells were analyzed on flow cytometry. Expression of MHC II, CD40, CD80, and CD86 on $CD11c^+$ cells is shown.