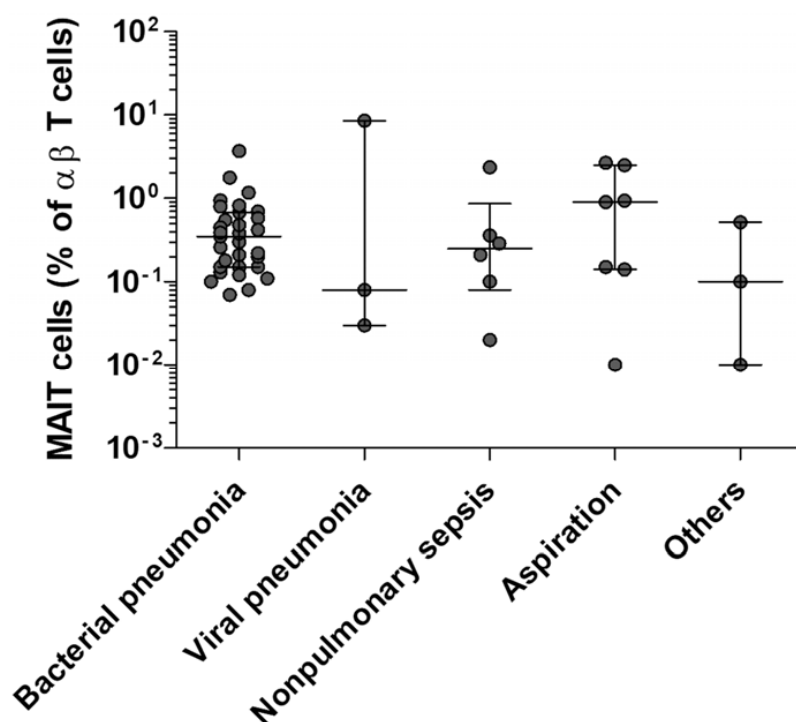
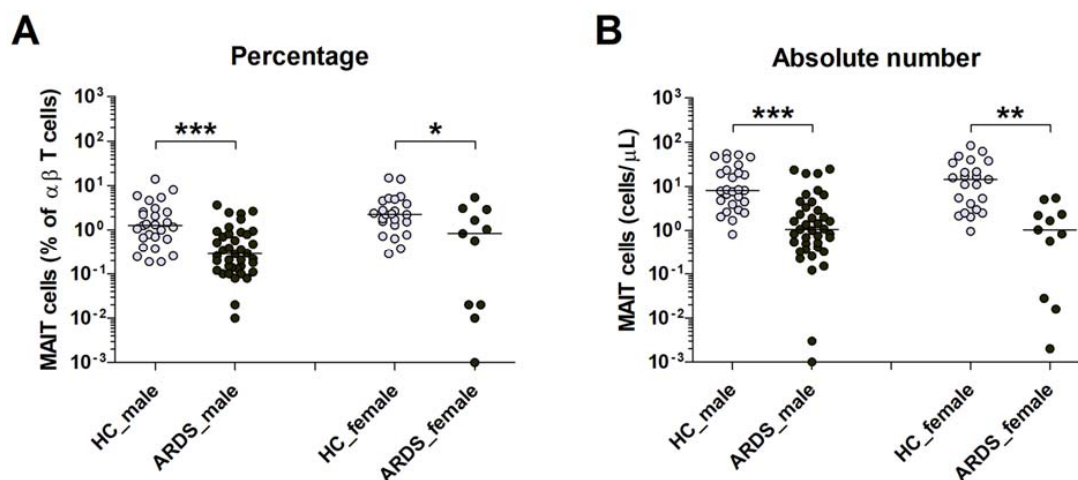


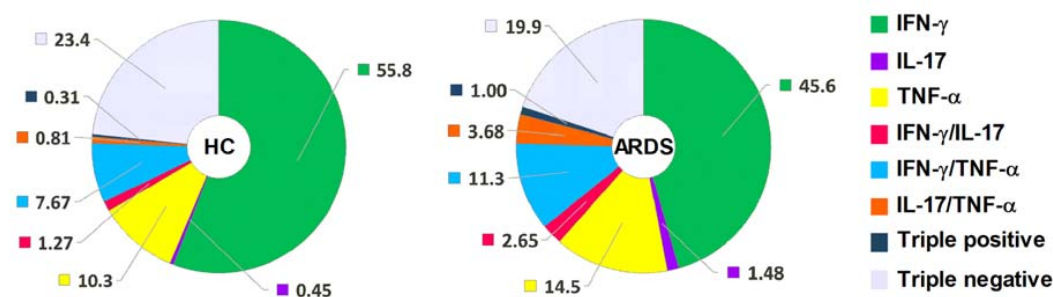
Supplementary figure S1 Isotype controls for flow cytometry of MAIT cells, cytokines, and activation. Freshly isolated PBMCs were stained with APC-Cy7-conjugated anti-CD3, FITC-conjugated anti-TCR $\gamma\delta$, APC-conjugated anti-TCR V α 7.2, PE-Cy5-conjugated anti-CD161, PE-conjugated anti-CD69, FITC-conjugated anti-IFN- γ , PE-conjugated anti-IL-17, PE-Cy7-conjugated anti-TNF- α , FITC-conjugated mouse IgG isotype, PE-conjugated mouse IgG isotype, PE-Cy5-conjugated mouse IgG isotype control, APC-conjugated mouse IgG isotype control, and PE-Cy7-conjugated mouse IgG isotype control mAbs and then analyzed by flow cytometry. Representative dot plots of (A) MAIT cell percentages, (B) IFN- γ , IL-17, and TNF- α expression in MAIT cells, and (C) CD69-expression in MAIT cells were compared with the corresponding isotype controls.



Supplementary figure S2 Frequency of circulating MAIT cells according to the cause of ARDS. Freshly isolated PBMCs from ARDS caused by bacterial pneumonia (n = 31), viral pneumonia (n = 3), nonpulmonary sepsis (n = 6), aspiration (n = 7) and others (n = 3) were stained with APC-Cy7-conjugated anti-CD3, FITC-conjugated anti-TCR $\gamma\delta$, APC-conjugated anti-TCR V α 7.2, and PE-Cy5-conjugated anti-CD161 mAbs and then analyzed by flow cytometry. Percentages of MAIT cells were calculated within a $\alpha\beta$ T cell gate. MAIT cell percentages among peripheral blood $\alpha\beta$ T cells. Symbols represent individual subjects and horizontal lines indicate the median value with the interquartile range. Statistical analysis was performed using Kruskal-Wallis test.



Supplementary figure S3 Frequencies of circulating MAIT cell numbers in the peripheral blood of ARDS patients by sex. Freshly isolated PBMCs from 27 male and 23 female HCs, 39 male and 11 female patients with ARDS were stained with APC-Cy7-conjugated anti-CD3, FITC-conjugated anti-TCR $\gamma\delta$, APC-conjugated anti-TCR V α 7.2, and PE-Cy5-conjugated anti-CD161 mAbs and then analyzed by flow cytometry. Percentages of MAIT cells were calculated within a $\alpha\beta$ T cell gate. (A) MAIT cell percentages among peripheral blood $\alpha\beta$ T cells. (B) Absolute MAIT cell numbers (per microliter of blood). Symbols represent individual subjects and horizontal lines are median values. * $p < 0.05$, ** $p < 0.0005$, *** $p < 0.0001$ by the Mann-Whitney U test.



Supplementary figure S4 Proportions of single-, double-, and triple-cytokine producing MAIT cells within HC and ARDS patients in the form of a pie-chart. Freshly isolated PBMCs (1×10^6 /well) from 20 HCs and 20 patients with ARDS were incubated for 1 hour in the presence of PMA (100 ng/ml) and IM (1 μ M). IFN- γ , IL-17, and TNF- α expression in the MAIT cell population was determined by intracellular flow cytometry. Expression pattern of MAIT cells producing the indicated cytokines on pie chart with color code. Values are expressed as mean.

Supplementary methods

Monoclonal antibodies (mAbs) and flow cytometry

The following mAbs and reagents were used in this study: allophycocyanin (APC)-Cy7-conjugated anti-CD3, phycoerythrin (PE)-Cy5-conjugated anti-CD161, fluorescein isothiocyanate (FITC)-conjugated anti-TCR $\gamma\delta$, FITC-conjugated anti-CD3, FITC-conjugated anti-IFN- γ , FITC-conjugated anti-TNF- α , PE-conjugated anti-IL-1 β , PE-conjugated anti-IL-8, PE-conjugated anti-IL-17, PE-Cy7-conjugated anti-TNF- α , PE-conjugated anti-CD69, PE-conjugated anti-lymphocyte-activation gene3 (anti-LAG3), FITC-conjugated mouse IgG isotype, PE-conjugated mouse IgG isotype, and PE-Cy7-conjugated mouse IgG isotype control (all from Becton Dickinson); PE-conjugated anti-programmed cell death-1 (anti-PD-1; eBioscience); APC-conjugated anti-TCR $V\alpha 7.2$ (BioLegend). Cells were stained with combinations of

appropriate mAbs for 20 minutes at 4°C. Stained cells were analyzed on a Navios flow cytometer using Kaluza software (Beckman Coulter).

Isolation of peripheral blood mononuclear cells (PBMCs) and identification of MAIT cells

Peripheral venous blood samples were collected in heparin-containing tubes and PBMCs were isolated by density-gradient centrifugation using Ficoll-Paque Plus solution (Amersham Bioscience). MAIT cells were identified phenotypically as CD3⁺TCR $\gamma\delta$ ⁻V α 7.2⁺CD161^{high} by flow cytometry as previously described.¹⁻³ Total lymphocyte numbers were measured with a Coulter LH750 automatic hematology analyzer (Beckman Coulter). Absolute numbers of MAIT cells were calculated by multiplying MAIT cell percentages by CD3⁺ $\gamma\delta$ ⁻ T cell percentages and total lymphocyte numbers (per microliter) in peripheral blood.

Functional MAIT cell assay

Expression levels of IFN- γ , IL-17 and TNF- α in MAIT cells were detected by intracellular cytokine flow cytometry as previously described.^{1 4-7} Freshly isolated PBMCs (1×10^6 cells/well) were incubated in 1 mL complete media consisting of RPMI 1640, supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 2 mM L-glutamine, 100 units/mL of penicillin, and 100 μ g/mL of streptomycin for 1 hours in the presence of phorbol myristate acetate (PMA; 100 ng/mL; Sigma) and ionomycin (IM; 1 μ M; Sigma). For intracellular cytokine staining, 10 μ L of brefeldin A (GolgiPlug; BD Biosciences) was added. The final concentration of brefeldin A was 10 μ g/mL. After incubation for an additional 4 hours, cells were stained with APC-Alexa Fluor 750-conjugated anti-CD3, PE-Cy5-conjugated anti-CD161, and APC-conjugated anti-TCR V α 7.2 mAb for 20 minutes at 4°C, fixed in 4% paraformaldehyde for 15 minutes at room

temperature, and permeabilized with Perm/Wash solution (BD Biosciences) for 10 minutes. Cells were then stained with FITC-conjugated anti-IFN- γ , PE-conjugated anti-IL-17, and PE-Cy7-conjugated anti-TNF- α mAbs for 30 minutes at 4°C and analyzed by flow cytometry.

Coculture system for human macrophage activity assay

Monocytes were differentiated into macrophage using coculture system as described previously.⁸ Briefly, monocytes were isolated from PBMCs at purities of > 95% using CD14 MicroBeads according to the instructions of the manufacturer (Miltenyi Biotec). Monocytes were seeded in a 6-well plate at 2.5×10^6 cells/well and cultured for 6 days in the presence of macrophage colony-stimulating factor (M-CSF; 100 ng/mL; PeproTech). Half medium was replaced at day 3 of culture. MAIT cells were isolated from PBMCs using a cell sorter (MoFlo Astrios; Beckman Coulter), and were activated in the presence of PMA (100 ng/mL; Sigma) and IM (1 μ M; Sigma) for 16 hours before coculture with macrophages. After 6 days, macrophages were cultured with resting MAIT cells (4×10^5 cells/well), or activated MAIT cells (4×10^5 cells/well) in the presence of M-CSF for 6, 12, 24, and 48 hours. After coculture, 10 μ L of brefeldin A (BD Biosciences) was added for intracellular cytokine staining. The final concentration of brefeldin A was 10 μ g/mL. After incubation for an additional 4 hours, the cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, and permeabilized with Perm/Wash solution (BD Biosciences) for 10 minutes. Cells were then stained with FITC-conjugated anti-TNF- α , PE-conjugated anti-IL-1 β , and PE-conjugated anti-IL-8 mAbs for 30 minutes at 4°C and analyzed by flow cytometry. Blocking antibodies for cytokines included anti-IL-17 (5 μ g/mL; R&D Systems), anti-IFN- γ (10 μ g/mL; BD biosciences) and anti-TNF- α (10 μ g/mL; BD biosciences). In the transwell systems, the activated MAIT cells and macrophages were seeded

respectively into the upper and lower compartment of the transwell with 0.4 μm pores (Corning Costar) in the presence of M-CSF.

Statistical analysis

All comparisons of percentages, absolute numbers, cytokine levels and ratios of MAIT cells, and expression levels of CD69, PD-1, and LAG3 were performed by analysis of covariance (ANCOVA) after adjusting for age and sex using Bonferroni correction for multiple comparisons. Linear regression analysis was used to test associations between MAIT cell levels and clinical or laboratory parameters. Wilcoxon matched-pairs signed rank test was used for comparison of total MAIT cell, CD69⁺ MAIT cell, and PD-1⁺ MAIT cell percentages and cytokine levels between peripheral blood and synovial fluid. Unpaired *t*-tests were used to compare changes in the expression of pro-inflammatory cytokines from human macrophages. Statistical significance was considered when p value was less than 0.05. All statistical analyses were performed using SPSS version 26.0 software (SPSS). GraphPad Prism version 5.03 software (GraphPad Software) was used for graphic works.

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