Supplementary Methods

Human Subjects

Study design and patient population. This study is an analysis of prospectively collected data from 39 subjects recruited from an ICU cohort from New York Presbyterian Hospital-Weill Cornell Medical Center (NYP-WMC). Subjects were recruited on the first or second day of their admission to the ICU. The cohort was derived from the Weill Cornell-Biobank of Critical Illness (WC-BOCI) at the NYP-WMC. Protocols for recruitment, data collection, and sample processing have been described previously [1-5]. Briefly, the WC-BOCI cohort recruited any patient admitted to the medical ICU, with exclusion for the lack of the ability to provide or the lack of a surrogate to provide informed consent and moribund state.

Clinical evaluation. Clinical and laboratory data were collected from the electronic health records by trained research personnel, with clinical adjudication of the final diagnosis of viral infection was confirmed by critical care board-certified attending physicians and clinical respiratory viral PCR (BioFire FilmArray RP2, BioFire Diagnostics, Salt Lake City, UT) was used to confirm viral etiology. ICU subjects without a concern for infection or in whom infection was not thought to be the cause of the current admission were used as the control population. Organ failure was defined by the SOFA scoring system, which quantifies the presence and severity of organ failure in 6 different organ systems [6].

Mice

Young adult (2 months) and aged adult (18 months) male and female BALB/c mice were purchased from the NIA rodent facility (Charles River Laboratories). Upon receipt, mice were handled under identical husbandry conditions and fed certified commercial feed. Body weights were measured daily and mice were humanely euthanized if they lost more than 15% of their

starting body weight. The IACUC at Weill Cornell Medicine approved the use of animals in this study and methods were carried out in accordance with the relevant guidelines and regulations. No animals were used in the study if there was evidence of skin lesions, weight loss, or lymphadenopathy.

Influenza (A/Puerto Rico/8/1934, H1N1)

Influenza viral stock (material #: 10100374, batch #: 4XP170531, EID_{50} per ml: $10^{10.3}$) was purchased from Charles River (Norwich, CT). $TCID_{50}$ was calculated using the Viral ToxGlo Assay (Promega, Madison WI). Briefly, BAL was diluted in 3.16-fold serial dilutions and plated for 24-48 hours on >80% confluent MDCK cells. Upon visualization of cytopathic effect, ATP detection reagent was added, and luminescence was measured. Values were calculated by plotting net relative luminescence units (RLU) values after subtracting average blank wells against viral dilution. The TCID50 value is the reciprocal of the dilution that produced a 50% decline in ATP levels compared to untreated controls. Validated regression analysis was performed using GraphPad Prism.

***In vivo* procedures and tissue collection**

Influenza infection: All mice were anesthetized with isoflurane (5% for induction and 2% for maintenance) prior to intranasal instillation with 12 PFU of influenza (50μL volume in PBS). Mitoquinol administration: Mice received a 100μL volume of PBS (vehicle) or 10-50μg dose of mitoquinol (Cayman Chemical) intraperitoneally starting at day 3 post influenza. BMS-986205 administration: Starting at day 0, mice received a daily intraperitoneal injection containing PBS or BMS-986205 (200μM) (Selleck Chemicals). Bronchoalveolar lavage (BAL): BAL was collected using previously published methods [7]. Briefly, 0.8-ml of PBS was slowly injected and aspirated 4 times prior to saving the recovered lavage fluid on ice. Lavage was clarified at 7000

rpm for 10 minutes at 4°C. Lung tissue collection: At select time points of infection lung tissue was collected from control and influenza infected young and aged adult mice. Tissue was snap frozen or placed into Allprotect (Qiagen) for future analysis. FITC-Dextran Lung Permeability Assay: Young and aged adult mice were intranasally instilled with 50-μL of FITC-Dextran (3mg/kg). After 1 hour, blood was collected from euthanized mice, and plasma was isolated after centrifugation (7000 rpm, 10 minutes). Fluorescence was assessed (excitation 485, emission 528). Lung Wet to Dry Ratio: Lung tissue was collected from control and influenza infected young and aged adult mice. Lung tissue weight was assessed at harvest (wet weight) and after being placed in a 60°C drying over for 48 hours (dry weight). Histology: Mice were euthanized, and right lung tissue was collected for downstream analysis. To maintain architecture, left lung was distended with 1% low melting agarose and placed into cold formalin [8]. Tissue samples were processed, and H&E stained by the Translational Research Program at WCM Pathology and Laboratory of Medicine. Images were scanned using the EVOS FL Auto Imaging System (ThermoFisher Scientific). For all animal experiments, we used 10 mice per group and experiments were repeated at least three times.

Primary bone marrow and alveolar macrophage isolation.

Bone marrow cells (BMCs) were prepared from the femurs and tibias of mice as previously described [9-11]. Alveolar macrophages were isolated from uninfected and influenza infected mice (day 3, 5, and 7) post instillation. Briefly, mice were lavaged with 5 x 1-ml of sterile PBS. Cells were collected and quantified.

***In vitro* cell culture treatments and assays.**

Bone marrow derived macrophages (BMM) were cultured with media alone or media containing LPS (Catalog #: tlrl-eblps, 100ng/ml) or poly I:C (HMW) (Catalog #: tlrl-pic, 1μg/ml) for 2, 4, or

24 hours (Invivogen, San Diego, CA). Aged BMM were treated with mitoTEMPO L (50μM) (Catalog #: 18796, Cayman Chemical), Trolox (50μM) (Catalog #: S3665, Selleck Chemicals, Houston TX), or mitoquinol (50μM) at time of plating (24h prior to stimulation). Young BMM were pre-treated with BMS-986205 (1μM) (Catalog #: S8629), NLG919 (1μM) (Catalog #: S7111), IDO inhibitor 1 (1μM) (Catalog #: S8557), Indoximod (1μM) (Catalog #: S7756) purchased from Selleck Chemicals) or 1-methyl-D_1-L-tryptophan (1μM) (Catalog #: ALX-106-040-M050, Enzo Life Sciences) 24 hours prior to stimulation. Young BMM were transfected with control or IDO1 specific siRNA (FlexiTube Gene Solution, Catalog #: GS15930, Qiagen) using GenMute siRNA transfection reagent for primary macrophages (Catalog #: SL100568-PMG, SignaGen Laboratories).

IDO1 specific activity assay

IDO1 specific activity was assessed using a standardized protocol (Catalog #: 9157-AO, R&D systems). Briefly, a substrate mixture (dilute ascorbic acid 80mM pH 8.0 mixed with 800μM L-tryptophan, 9000 units/mL catalase, and 40μM methylene blue in 50mM MES pH 6.5). Dilute rmIDO (8ng/μL) was used as a positive control. The reaction was started by adding 50μL of substrate mixture to 50μL of substrate blank, positive control, or sample and absorbance at 321 nm was measured and IDO1 specific activity was calculated: Specific Activity (pmol/min/mg) = [Adjusted V_{max} (OD/min) X well volume (L) x 10^{12} pmol/mol] / [extinction coefficient (3750 M^{-1} cm^{-1}) x pathway correction 0.32 cm x IDO1 concentration (μg)]. To confirm these results, samples were also assessed by fluorescence using the IDO1 enzyme assay kit (Catalog #: K972, Biovision).

Flow cytometry

Mitochondrial superoxide generation was detected using MitoSOX red indicator (Catalog #: M36008, ThermoFisher Scientific). Cells were loaded with 1ml of 5 μM MitoSOX and incubated for 10 minutes at 37°C. Cells were washed and analyzed. All samples were run on a BD Accuri and analyzed by Flow Jo software (Tree Star Inc., Ashland, OR).

NAD^+ Assay

Lung tissue was collected from control and influenza infected young and aged adult BALB/c and NAD^+ levels were assessed using the NAD/NADH Quantitation Colorimetric Kit (Biovision, Catalog# K337).

ELISA

Culture supernatants, lung homogenates, and BAL were analyzed for IL1β, IL10, TNFα, and IL6 production using ELISA kits purchased from Thermo Fisher Scientific. Kynurenine levels were measured by ELISA (Catalog #: E4629, BioVision) and repeated with sample acylation and detection (Catalog #: ISE-2227, Immusmol, Bordeaux, France). Tryptophan was quantified by fluorometric assessment (Catalog #: K557, BioVision) and repeated with precipitation and derivation prior to ELISA detection (Catalog #: ISE-2227, Immusmol). Protein levels were calculated using the BioRad protein assay (BioRad) per manufacturer's instructions.

$CD4^+CD25^+$ Cell Isolation

Lung tissue was homogenized using a glass dounce homogenizer to form single-cell suspensions prior to isolation using the EasySep Mouse $CD4^+CD25^+$ Regulatory T Cell Isolation Kit II (Catalog #: 18783, STEMCELL Technologies). $CD4^+$ T cells were first enriched by negative selection using the mouse $CD4^+$ T cell isolation cocktail followed by positive selection for $CD25^+$ T cells. Cells were stained with trypan blue and enumerated.

Metabolite Sample Accessioning: Following receipt, samples were inventoried and immediately stored at -80^{o}C. Each sample received was accessioned into the Metabolon LIMS system and was assigned by the LIMS a unique identifier that was associated with the original source identifier only. This identifier was used to track all sample handling, tasks, results, etc. The samples (and all derived aliquots) were tracked by the LIMS system. All portions of any sample were automatically assigned their own unique identifiers by the LIMS when a new task was created; the relationship of these samples was also tracked. All samples were maintained at -80^{o}C until processed.

Metabolite Sample Preparation: Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis. Several types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated by taking a small volume of each experimental sample (or alternatively, use of a pool of well-characterized human plasma) served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards that were carefully chosen not to interfere with the measurement of endogenous compounds were spiked into

every analyzed sample, allowed instrument performance monitoring and aided chromatographic alignment. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections. Peaks were quantified using area-under-the-curve. A data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Essentially, each compound was corrected in run-day blocks by registering the medians to equal one (1.00) and normalizing each data point proportionately. In certain instances, biochemical data may have been normalized to an additional factor (e.g., cell counts, total protein as determined by Bradford assay, osmolality, etc.) to account for differences in metabolite levels due to differences in the amount of material present in each sample.

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS): All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed

using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same afore mentioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MS^n scans using dynamic exclusion. The scan range varied slighted between methods but covered 70-1000 m/z.

Bioinformatics: The informatics system consisted of four major components, the Laboratory Information Management System (LIMS), the data extraction and peak-identification software, data processing tools for QC and compound identification, and a collection of information interpretation and visualization tools for use by data analysts. The hardware and software foundations for these informatics components were the LAN backbone, and a database server running Oracle 10.2.0.1 Enterprise Edition.

Metabolite Quantification and Data Normalization: Peaks were quantified using area-under-the-curve. For studies spanning multiple days, a data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. Following log transformation and imputation of missing values, if any, with the minimum observed value for each compound, Welch's two-sample t-test was used to identify biochemicals that differed significantly between experimental groups. Biochemicals that achieved statistical significance

($p \leq 0.05$) were presented. In additional, Spearman Correlation was used as a nonparametric measure of the strength and direction of association that exists between two variables.

Principal Components Analysis (PCA)

Principal components analysis is an unsupervised analysis that reduces the dimension of the data. Each principal component is a linear combination of every metabolite and the principal components are uncorrelated. The number of principal components is equal to the number of observations. The first principal component is computed by determining the coefficients of the metabolites that maximizes the variance of the linear combination. The second component finds the coefficients that maximize the variance with the condition that the second component is orthogonal to the first. The third component is orthogonal to the first two components and so on. The total variance is defined as the sum of the variances of the predicted values of each component (the variance is the square of the standard deviation), and for each component, the proportion of the total variance is computed.

RNA purification and real time PCR

RNA samples were extracted using the automated Maxwell RNA extraction protocol (Madison, WI). Samples were quantified and $A_{260/280}$ ratios were recorded. Samples were reverse transcribed using the First Stand Synthesis Kit and quantified using QuantiTect Primer Assays and RT^2 Profiler™ Assays (Mouse Mitochondria PAMM-0087Z) were used to assess gene expression (Qiagen). For Taqman assays, RNA was reverse transcribed using prior to assessment using Taqman Fast Advanced Master Mix and probes specific for metabolic genes (Mouse Amino Acid Metabolism, catalog #: 4391524) or genes associated with immune responses (Mouse Immune Response, catalog #: 4414079). All reactions were performed in triplicate. Relative levels of messenger RNA (mRNA) were calculated by the comparative cycle

threshold method and either β-Actin or β2M mRNA levels were used as the invariant control for each sample.

Statistical analysis: Murine Studies

Survival analysis between groups was calculated using the Mantel Cox test. Comparison of groups was performed using a two-tailed t-test and comparisons between groups were verified by one-way ANOVA. All samples were independent and contained the same sample size for analysis. All data were analyzed using GraphPad Prism software (San Diego, CA). Statistical significance was considered by a *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

Data availability

Most data generated during this study are included in this published article and its Supplementary Information files. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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