Appendix 1 Supplementary methods

Basic anesthesia and ex vivo lung perfusion (EVLP) protocol

Rats were subjected to isoflurane inhalation (Hana Pharm, Seoul, Korea), followed by tracheotomy and mechanical ventilation with O_2 and 3% isoflurane. A 20-mL preservation solution (Perfadex Plus; XVIVO, Göteborg, Sweden) containing 3 µg prostaglandin E1 (Alpostin; Dongkook Pharmaceutical, Seoul, South Korea) was infused through the pulmonary artery, after which the lungs were harvested. The lung grafts were procured and preserved at 4 ℃ for 1 h. After cannulation and during cold ischemia, EVLP was applied for 4 h according to a previously published protocol (22). Briefly, EVLP was performed using a commercially available rodent system [interleukin (IL)-2 isolated perfused rat or guinea pig lung system; Harvard Apparatus, Holliston, MA, USA] (23). EVLP for basic experiments was performed in the same manner as described previously (23). During EVLP, the lungs were ventilated with air; perfused with STEEN solution (XVIVO Perfusion AB, Göteborg, Sweden), deoxygenated with 6% O_2, 8% CO_2, and balanced N_2; and supplemented with 50 mg methylprednisolone (Solu-Medrol; Pfizer, Inc., NY, USA) and 50 mg cephalosporin (Cefazolin; West-Ward Pharmaceuticals Corp., Eatontown, NJ, USA). Ventilation was set to a pressure-controlled mode (15 cmH_2O) with 5 cmH_2O positive end-expiratory pressure and a respiratory rate of 30 breaths/min. Perfusion flow was initiated when 10% of the target flow was achieved and then gradually increased for 1 h to a target flow rate calculated as 20% of the cardiac output (75 mL/min/250 g body weight). There was no humane endpoint because this study involved ex vivo experiments.

Sample preparation for capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS)

The samples for each group (sham, control, and study) were placed in a homogenization tube with zirconium beads (5 mm φ and 3 mm φ); 750 µL of 50% acetonitrile in Milli-Q water (v/v) containing internal standards (20 µM) were added to the tube. Using a bead shaker, the samples were completely homogenized at 1,500 rpm at 4 ℃, four times for 2 min each. Subsequently, 750 µL of 50% acetonitrile in Milli-Q water (v/v) was added to the mixture, and the samples were homogenized again. The homogenate was centrifuged at 2,300 ×g at 4 ℃ for 5 min, and the upper aqueous layer was centrifugally filtered at 4 ℃ through a 5-kDa cutoff filter (ULTRAFREE-MC-PLHCC; Human Metabolome Technologies, Yamagata, Japan) for macromolecule removal. The filtrate was evaporated to dryness under vacuum and reconstituted in Milli-Q water for CE-TOFMS analysis.

CE-TOFMS conditions

Cationic metabolites were analyzed using a fused silica capillary column (i.d. 50 µm × 80 cm) with commercial cation electrophoresis buffer (Solution ID: H3301-1001, Human Metabolome Technologies). The sample was injected at a pressure of 50 mbar for 10 s at an applied voltage of 30 kV. Electrospray ionization-mass spectrometry (ESI-MS) was conducted in the positive-ion mode with a capillary voltage of 4000 V. The spectrometer scanned the range of mass-to-charge ratio (*m/z*) of 50–1000.

Anionic metabolites were analyzed using a fused silica capillary column (i.d. 50 µm × 80 cm) with commercial anion electrophoresis buffer (Solution ID: I3302-1023, Human Metabolome Technologies). The sample was injected at a pressure of 50 mbar for 10 s at an applied voltage of 30 kV. ESI-MS was conducted in the negative ion mode with a capillary voltage of 3500 V. The spectrometer scanned the range of *m/z* ratio of 50–1000.

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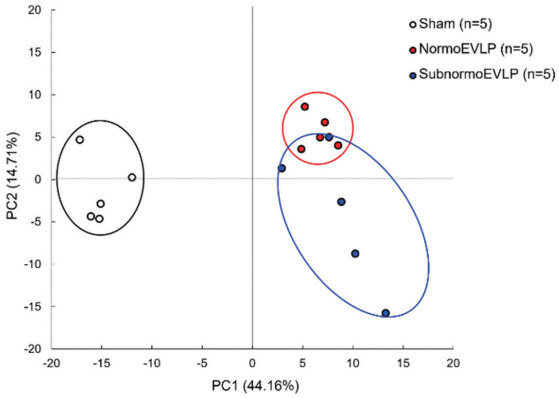

Figure S1 PCA. The plot indicates the PCA score for each sample. The percentages represent the contribution rate of each component. EVLP, ex vivo lung perfusion; PCA, principal component analysis.

Table S1 Primer sequences for qRT-PCR

Symbol	Forward	Reverse
TNF-α	AAGCTGTCTTCAGGCCAACA	CCCGTAGGGCGATTACAGTC
IL-1β	GTCTGACCCATGTGAGCTGAA	CAAGGCCACAGGGATTTTGTC
IL-6	TAGTCCTTCCTACCCCAACTTCC	TTGGTCCTTAGCCACTCCTTC
IL-18	TGGAATCAGACCACTTTGGCA	TCTGGGATTCGTTGGCTGTT
HK	GACGAACCTGGACTGTGGAAT	TCCTCTCTCCTCTTCACCGC
PFK	ATCCACGACTTGAAGGCCAA	CTGCAGTCGAACACACCTCT
PK	CCTGATAGCTCGAGAGGCTG	TATAAGAGGCCTCCACGCTG
HIF-1α	ACATCTTCTTCTGCTCCACTAC	CTGGAGATTAGTAATGGCCCAT
mTORC	ACTGTTCCTGTCCATGTA TCTG	GTAGTGGAGCAGAAGAAGATGT
NLRP3	GCCACTATGTACTCAT ACGACA	AGTCAGGGATCTTCACTTTGAG
Caspase-1	AAAGATTCAGTAGGGAACTCCG	TCACAAGACCAGGCATATTCTT
GAPDH	TCTCTGCTCCTCCCTGTTCTA	ATGAAGGGGTCGTTGATGGC

qRT-PCR, quantitative reverse transcription-polymerase chain reaction; TNF-α, tumor necrosis factor-α; IL, interleukin; HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; HIF-1α, hypoxia-inducible factor 1α; mTORC, mammalian target of rapamycin complex; NLRP3, nucleotide-binding domain, leucine-rich-containing family pyrin domain containing 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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Table S2 Metabolites showing the 10 highest and lowest PCA loading factors

Rank	Component 1 (44.16%)		Component 2 (14.71%)	
	Metabolite	PCA loading	Metabolite	PCA loading
Highest				
1	Octanoic acid	0.98997	Choline	0.92145
2	N-Acetyltryptophan	0.97772	UDP-N-acetylgalactosamine; UDP-N-acetylglucosamine	0.91567
3	Ibuprofen	0.96209	Glucose 1-phosphate	0.87656
4	o-Hydroxybenzoic acid	0.95795	XA0065	0.82838
5	Butyric acid; Isobutyric acid	0.95366	γ-Glu-Gly	0.81370
6	Glycerol	0.91829	Inosine	0.80679
7	8-Hydroxyoctanoic acid-1; 2-Hydroxyoctanoic acid-1	0.89003	Uridine	0.80466
8	Hexanoic acid	0.88520	Guanosine	0.80181
9	N^1-Acetylspermidine	0.86887	CMP	0.78705
10	XC0154	0.86246	N-Acetylglucosamine 1-phosphate	0.77429
Lowest				
1	Leu	-0.99215	4-Methyl-2-oxovaleric acid; 3-Methyl-2-oxovaleric acid; 2-Oxohexanoic acid	-0.72633
2	Met	-0.98692	Thiaproline	-0.68469
3	S-Methylglutathione	-0.98601	2-Oxoisovaleric acid	-0.66474
4	Ile	-0.98495	Phosphoenolpyruvic acid	-0.63699
5	Val	-0.97747	Pyruvic acid	-0.63316
6	Pro	-0.97709	Phosphocreatine	-0.55766
7	Homoserine lactone	-0.97108	2-Oxoglutaric acid	-0.54713
8	N, N-Dimethylglycine	-0.96416	Isoglutamic acid	-0.51861
9	S-Adenosylmethionine	-0.96004	Putrescine	-0.50646
10	S-Lactoylglutathione	-0.95747	1-Methylnicotinamide	-0.48822

PCA, principal component analysis.

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