Supplemental Digital Content 4

Histological Measurements

Following ¹⁸F-FDG imaging, animals were euthanized and the lungs were removed and fixed at a pressure of 25 cmH₂O with Trump's fixative in 4 High-Strain animals, 5 High-Strain LPS animals, and 6 Low-Strain LPS animals. Tissue samples were cut from ventral and dorsal regions, sectioned with 5 μm thickness, and mounted for hematoxylin-eosin staining. Using light microscopy with 400× magnification, two blinded investigators evaluated neutrophil counts within 10 randomly selected fields, as well as the following indices on a semi-quantitative scale: alveolar edema, alveolar hemorrhage, septal thickening, and septal congestion (none=0, 1=mild, 2=moderate, 3=marked).

Neutrophilic Infiltration and Parenchymal Injury

Tissue histology revealed a significant increase in tissue neutrophil counts resulting from LPS exposure in both non-dependent and dependent regions (Figure S9), with similar neutrophil counts between the LPS groups in both regions (Table S2). Alveolar edema was absent or mild in both regions for all groups, and indices of injury were generally low and without differences between groups (Table S2), indicating a very mild degree of tissue injury.

Table S2. Histologic Measures of Regional Neutrophilic Infiltration and Injury

	High-Strain		High-Strain LPS		Low-Strain LPS	
	Non-Dep.	Dependent	Non-Dep.	Dependent	Non-Dep.	Dependent
Neutrophil count	2.9 ± 1.3	5.9 ± 1.5	7.4 ± 2.3	11.7 ± 3.2	11.1 ± 5.5	15.8 ± 5.2
Alveolar edema	0 (0-0)	1 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Alveolar hemorrhage	0 (0-0)	1 (1-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Septal thickening	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-2)	1 (1-2)
Congestion	1 (1-1)	1 (1-2)	1 (0-1)	0 (0-1)	1 (0-1)	1 (0-1)

Shown are mean±SD or median (25th to 75th percentile) of 10 randomly selected fields from non-dependent (Non-Dep) and dependent regions studied at 400x magnification in 4 High-Strain, 5 High-Strain LPS, and 6 Low-Strain LPS animals.

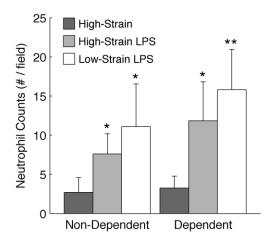


Figure S9. Regional neutrophil counts in 400x fields assessed from histology of non-dependent (ventral) and dependent (dorsal) tissue samples. LPS exposed groups showed significantly higher neutrophil counts in both regions compared to the unexposed group, with no significant differences between the LPS groups in either region. *p<0.05, **p<0.01 vs. High-Strain in same region.