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Supplementary Materials for

Imaging *Enterobacterales* infections in patients using pathogen-specific positron emission tomography

Alvaro A. Ordonez, Luz M. Wintaco, Filipa Mota, Andres F. Restrepo, Camilo A. Ruiz-Bedoya, Carlos F. Reyes, Luis G. Uribe, Sudhanshu Abhishek, Franco R. D'Alessio, Daniel P. Holt, Robert F. Dannals, Steven P. Rowe, Victor R. Castillo, Martin G. Pomper, Ulises Granados*, Sanjay K. Jain*

*Corresponding author. Email: ulisesgranados@fcv.org (U.G.); sjain5@jhmi.edu (S.K.J.)

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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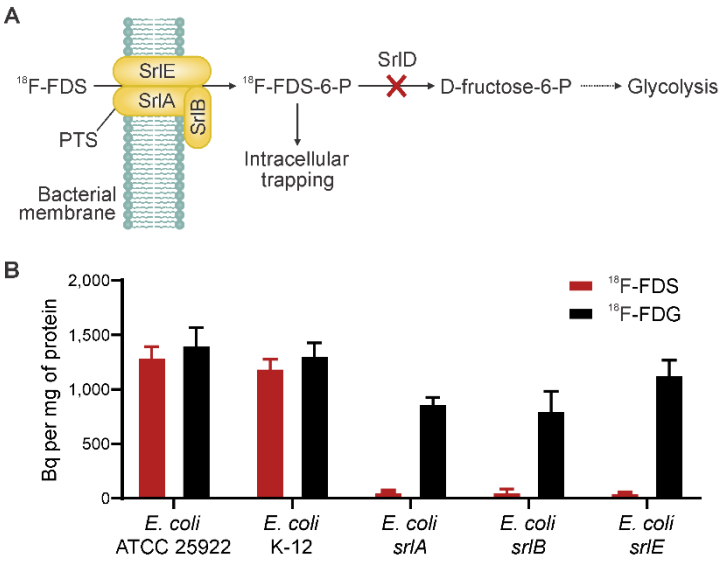

Fig. S1. Mechanism of ^{18}F-FDS accumulation in bacteria. (**A**) *Enterobacterales* can utilize sorbitol as a sole carbon source. ^{18}F-FDS uptake in *Enterobacterales* occurs via a metabolically conserved pathway mediated by a sorbitol-specific phosphoenolpyruvate-dependent, sugar-transporting phosphotransferase system (PTS) (*9*). In *E. coli*, PTS is composed of three subunits: SrlE, SrlA and SrlB. The *srl* operon is induced by sorbitol, and PTS transports sorbitol with low micromolar affinities (*10*). Sorbitol 6-phosphate is then oxidized by sorbitol-6-phosphate 2-dehydrogenase (SrlD) into D-fructose 6-phosphate which is subsequently metabolized, primarily via glycolysis. ^{18}F-FDS 6-phosphate is not recognized as a substrate by the highly specific bacterial SrlD and is trapped inside the bacteria. (**B**) In vitro uptake of ^{18}F-FDS and ^{18}F-FDG at 120 min in *E. coli* ATCC 25922 (reference strain), *E. coli* K-12 (parent strain) and *E. coli* K-12 *srlA*, *srlB*, and *srlE* knockout strains. Data represented as mean and standard deviation (n = 5 replicates for each bacterial strain).

Fig. S2. Whole body ^{18}F-FDS PET/CT. Three-dimensional maximum intensity projections 1 h after tracer injection are shown for all 26 patients. The CT is represented in blue, while the ^{18}F-FDS PET signal is represented in orange-red. All images are represented using the same standardized uptake value (SUV) scale (0-5).

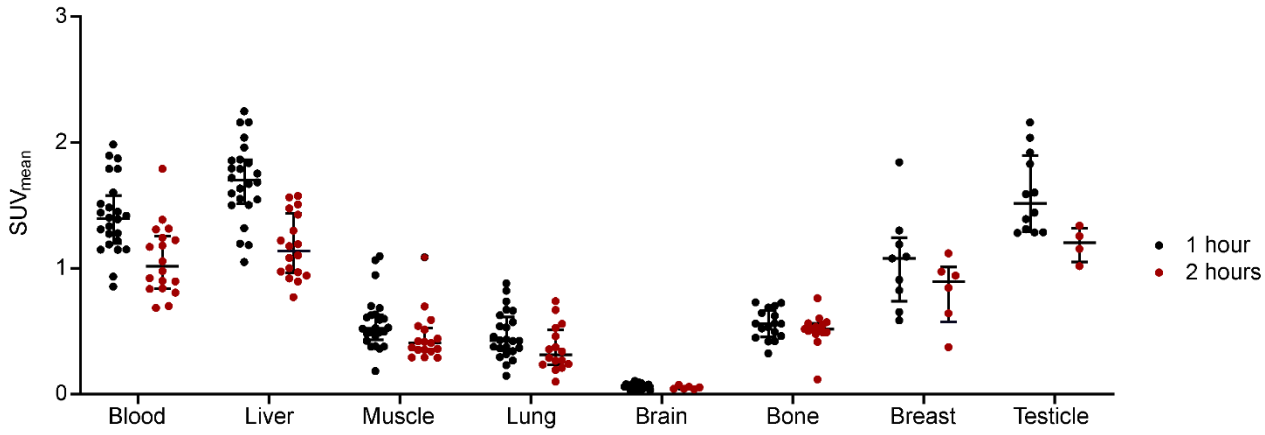

Fig. S3. Biodistribution of ^{18}F-FDS in unaffected tissues. The mean standardized uptake value (SUV_{mean}) derived from ^{18}F-FDS PET for all 26 patients at 1 h (black) and 2 h (red) after tracer injection is shown. Data is represented as median and IQR.

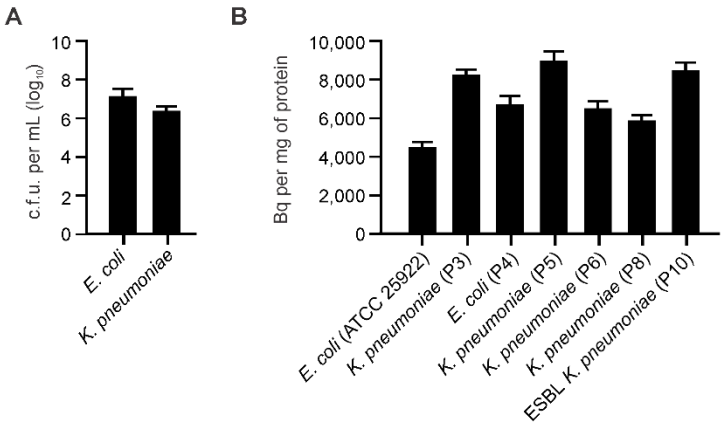

Fig. S4. Bacterial burden and in vitro ^{18}F-FDS uptake. (**A**) Bacterial burden in biological samples collected from patients with *E. coli* or *K. pneumoniae* infections. Data represented as mean and standard deviation (n = 3 isolates for each strain). (**B**) In vitro ^{18}F-FDS uptake in *Enterobacterales* strains (including one MDR, ESBL-producing strain) isolated from infected patients from this study compared to a reference strain (*E. coli* ATCC 25922). Data represented as mean and standard deviation (n = 6 replicates for each strain).

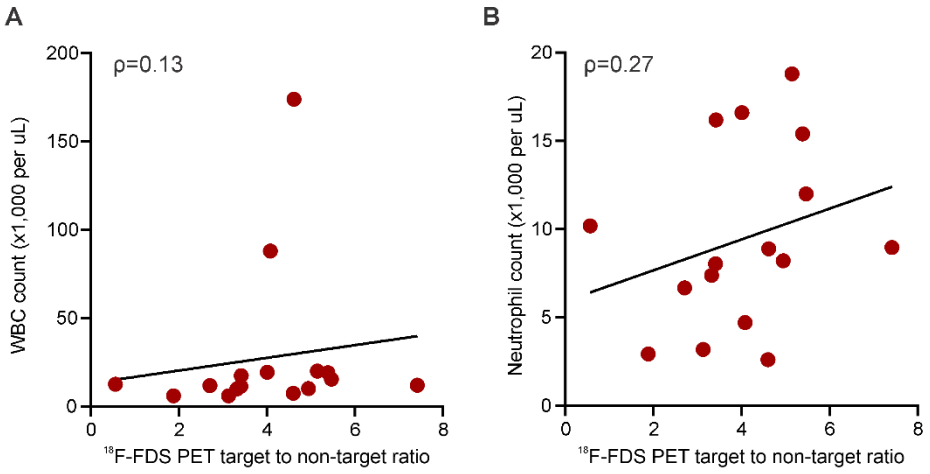

Fig. S5. Correlation of ^{18}F-FDS PET with host inflammatory cells. Correlation of ^{18}F-FDS PET target to non-target ratio 2 h post-injection with (**A**) peripheral white blood cell (WBC) counts (Pearson ρ = 0.13, *P* = 0.62) and (**B**) peripheral neutrophil counts (Pearson ρ = 0.27, *P* = 0.30).

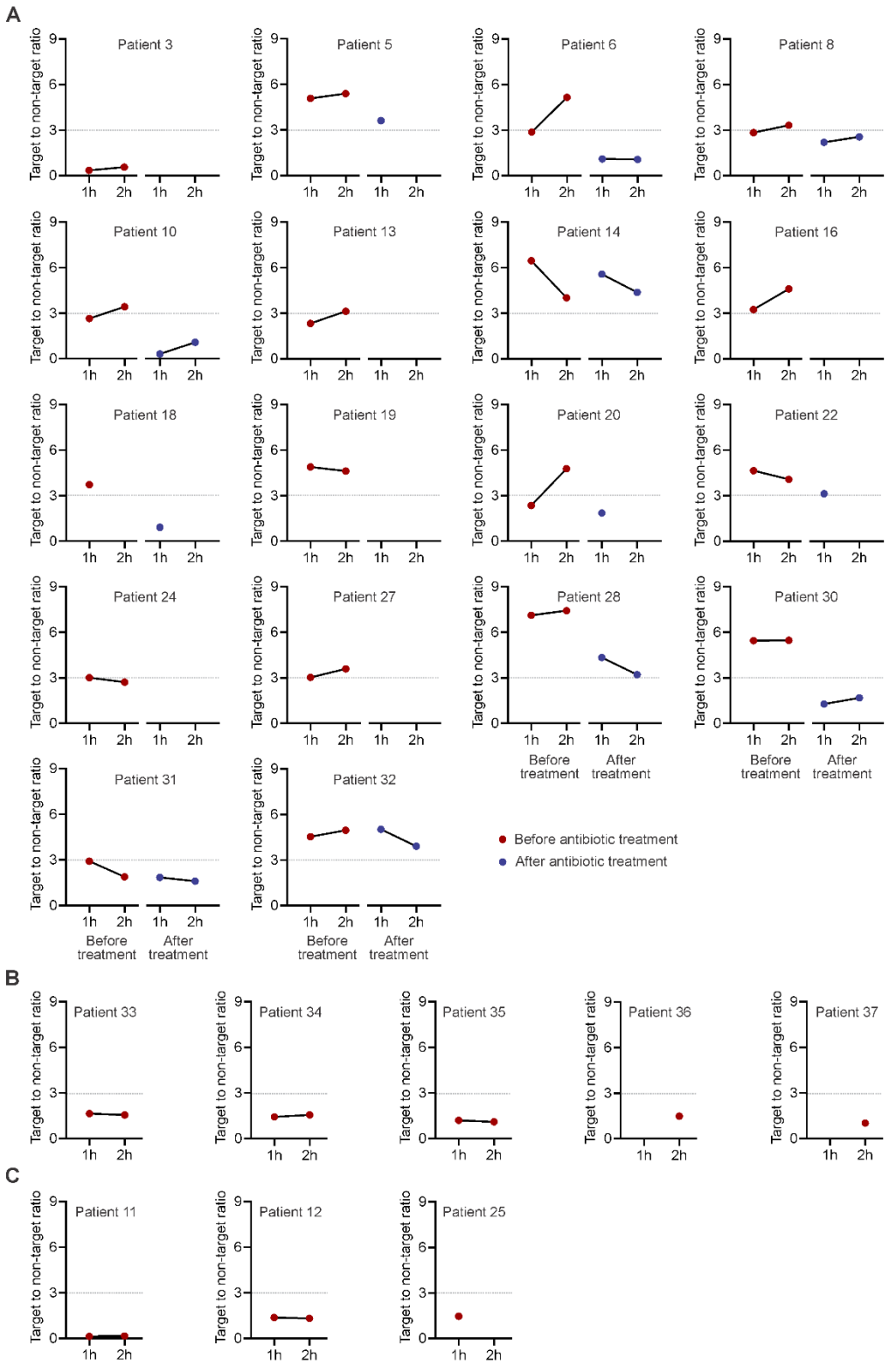

Fig. S6. ^{18}F-FDS PET target-to-nontarget ratios for all patients. ^{18}F-FDS PET target-to-non-target ratio at 1 and 2 h after tracer injection are shown for all 26 patients. (**A**) Microbiologically confirmed *Enterobacterales* infection, and the subset of patients imaged again after completion of antibiotic treatment. (**B**) Confirmed inflammatory or oncological disease and (**C**) microbiologically confirmed infections due to non-*Enterobacterales*. The dotted line indicates the target-to-nontarget ratio cut off of 3, determined in this study to be indicative of infection.

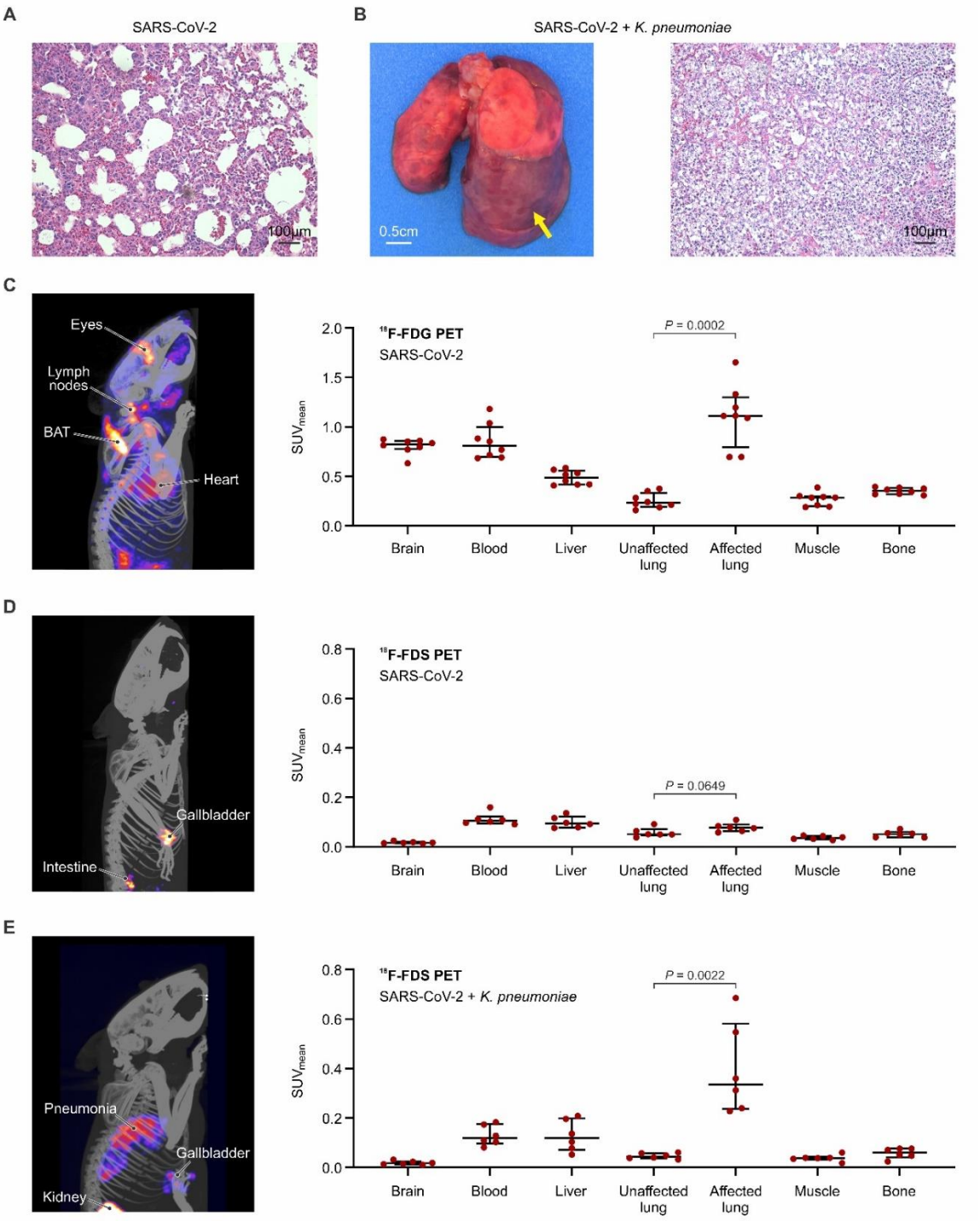

Fig. S7. Hamster model pathology. (**A**) Hematoxylin and eosin (H&E) staining of lungs from a representative SARS-CoV-2-infected hamster 7 days post-infection. (**B**) Gross pathology (yellow arrow indicates affected area) and histology (H&E) of the lungs of a hamster infected with SARS-CoV-2 and *K. pneumoniae*. (**C**) Three-dimensional maximum intensity projection (MIP) of an animal infected with SARS-CoV-2 and imaged with ^{18}F-FDG and corresponding tissue biodistribution for all animals (n = 8) represented as mean standardized uptake value (SUV_{mean}). (**D**) ^{18}F-FDS PET/CT MIP of an animal infected with SARS-CoV-2 and corresponding tissue biodistribution for all animals (n = 6). (**E**) ^{18}F-FDS PET/CT MIP of an animal infected with SARS-CoV-2 and *K. pneumoniae* and corresponding tissue biodistribution for all animals (n = 6). Data is represented as median and IQR. Statistical comparisons performed using a two-tailed Mann-Whitney *U* test. BAT, brown adipose tissue.

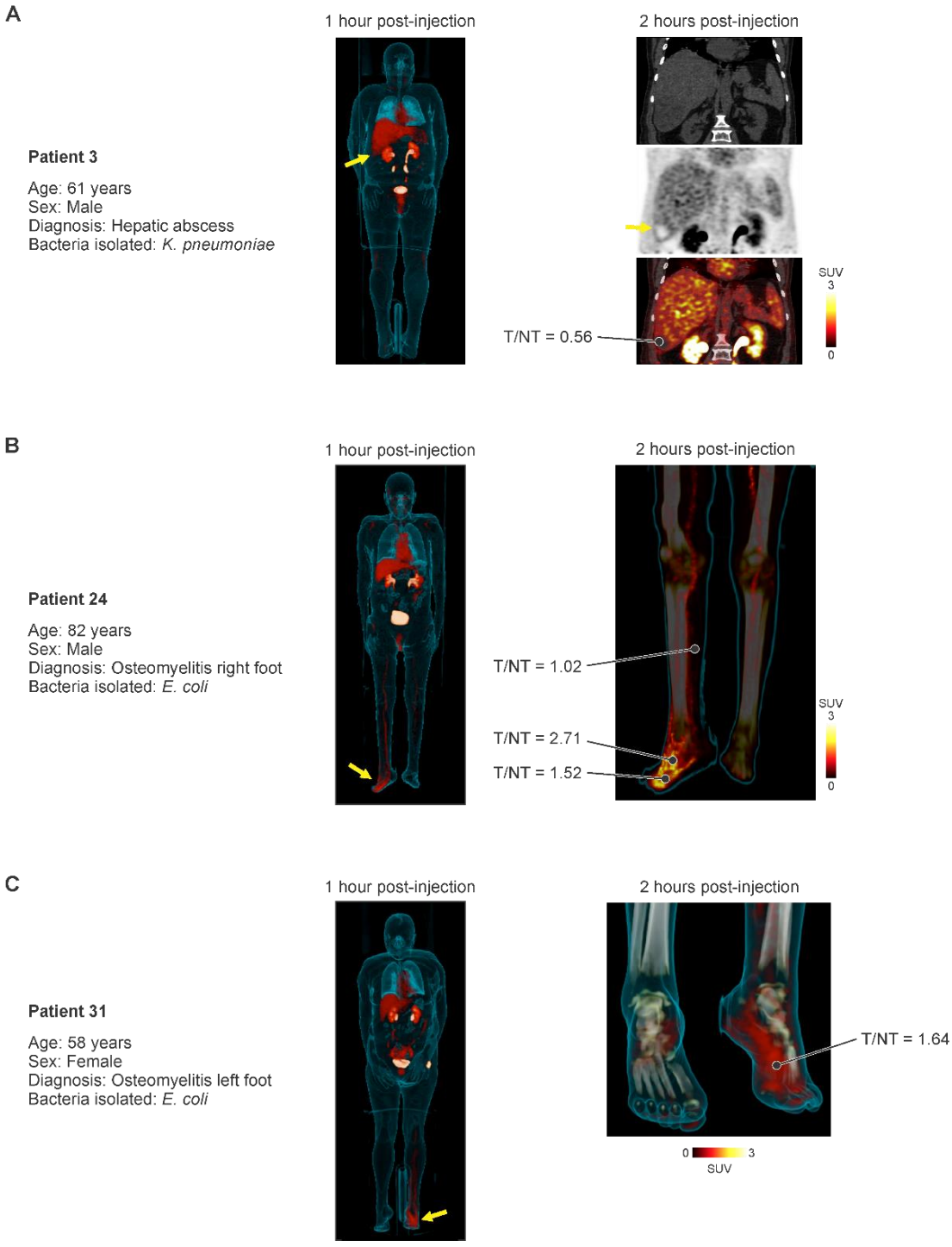

Fig. S8. Patients with false-negative ^{18}F-FDS PET. (**A**) Three-dimensional maximum intensity projection (MIP) shown on the left, coronal CT (top), PET (middle), and overlaid PET/CT (bottom) shown on the right from 61-year-old male presenting with a hepatic abscess due to *K. pneumoniae* with a focus of photopenia (arrow). (**B** and **C**) Three-dimensional MIP for whole body (left panels) and localized site of infection (right panels) from two patients with diabetes with microbiologically confirmed foot osteomyelitis. While a higher ^{18}F-FDS signal can be visually assessed at the infection site, the target-to-nontarget tissue ratio (TNT) is below the cutoff of 3. The yellow arrows point to the site of infection.

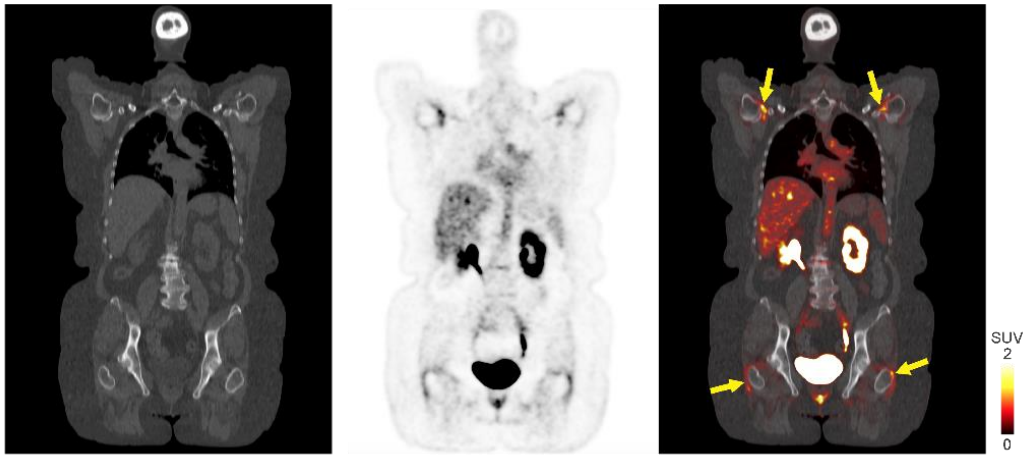

Fig. S9. ^{18}F-FDS accumulation in fluids. Coronal CT (left), PET (middle), and overlaid PET/CT (right) from a patient with interstitial lung disease. ^{18}F-FDS PET signal was observed in the synovial fluid (yellow arrows).

Table S1. Selection criteria for patient enrollment in the clinical study.

- Greater than or equal to 18 years old
- Patients with microbiologically confirmed *Enterobacterales* infection OR with high suspicion for *Enterobacterales* infection with microbiological-confirmation anticipated within 72 hours of imaging:
    - Received no or ≤ 72 hours of antibiotic treatment for the current episode of infection by the time of first ^{18}F-FDS PET/CT
    - Microbiological-confirmation was defined as the isolation of bacteria from a sample obtained directly from the infection site
    - Patients with suspected infection without microbiological-confirmation were excluded from the study analysis
- Control patients: Confirmed inflammatory or oncologic disease and clinically determined not to have an infection
- Determined by the patient's medical team to be stable to participate in the study
- Not pregnant
- Serum creatinine < 3 times the upper limit of normal
- Total bilirubin < 3 times the upper limit of normal
- Liver transaminases < 5 times the upper limit of normal
- Not treated with an investigational drug, biologic, or therapeutic device within 30 days prior to the ^{18}F-FDS PET/CT
- Adequate venous access
- Ability to provide written informed consent

Table S2. Patient characteristics.

ID	Age (years)	Sex	Weight (kg)	Diagnosis	Comorbidities	Pathogen isolated	Sample obtained from	Neutrophil count ($x10^3/\mu L$)	Notes
1	54	F	53	Brain abscess	Chagas disease	No sample	-	7.20	Excluded
2	69	M	74	Pneumonia	DM	*Raoultella planticola*	BAL	6.07	Excluded
3	61	M	84	Hepatic abscess	DM, CKD	*Klebsiella pneumoniae*	Abscess drainage	10.20	
4	44	F	60	Peritonitis	Abdominal trauma	*Escherichia coli* and *Klebsiella pneumoniae*	Peritoneal fluid	5.81	Excluded
5	67	M	59	Pneumonia	Lung cancer	*Klebsiella pneumoniae*	BAL	15.40	
6	35	F	69	Abdominal abscess	Gastric cancer	*Klebsiella pneumoniae*	Abscess drainage	18.80	
7	33	M	55	Abdominal collection	Appendicitis, post-surgery	Negative cultures	Collection drainage	8.64	Excluded
8	70	M	70	Pleural empyema	-	*Klebsiella pneumoniae*	BAL	7.39	
9	63	F	73	Hepatic abscess	DM, CKD	*Escherichia coli*	Abscess drainage	12.40	Excluded
10	38	M	69	Intracranial infection	Urachal cancer	ESBL *K. pneumoniae*	CSF and wound drainage	16.20	
11	31	M	65	Hepatic abscess	-	*Entamoeba histolytica*	Abscess drainage	11.90	
12	35	F	35	Bronchitis / pneumonia	Asthma	*Pseudomonas aeruginosa*	BAL	3.88	
13	60	F	42	Bronchiectasis exacerbation	-	*Klebsiella pneumoniae*	BAL	3.19	
14	33	M	63	Infected bone fracture	Leg trauma	ESBL *E. coli*	Tissue sample	16.60	
15	70	M	73	Fournier gangrene	DM	*Klebsiella pneumoniae*	Tissue sample	15.40	Excluded
16	65	F	92	Cellulitis right arm	Breast cancer	*Klebsiella aerogenes*	Tissue sample	2.62	
17	70	M	57	Sacral ulcer	DM	*Escherichia coli*	Tissue sample	17.40	Excluded
18	69	M	65	Pneumonia	COPD	*Klebsiella pneumoniae*	BAL	15.20	
19	64	M	80	Pneumonia and aspergillosis	AML	*Enterobacter cloacae*	BAL	8.89	
20	78	M	60	Pneumonia	Gastric cancer	*Klebsiella pneumoniae*	BAL	9.36	

21	74	M	84	Abdominal collection	Nephrectomy	*Citrobacter freundii* and *Klebsiella pneumoniae*	Collection drainage	7.81	Excluded
22	59	M	83	Ischiorectal abscess	Fournier gangrene	ESBL *E. coli*	Tissue sample	4.72	
23	68	M	83	Pneumonia	Lung cancer	Negative cultures	Lung biopsy	11.20	Excluded
24	82	M	68	Osteomyelitis right foot	DM, PAD	*Escherichia coli*	Tissue sample	6.68	
25	74	F	52	Pneumonia	Osteoporosis	*Mycobacterium tuberculosis*	BAL	3.81	
26	53	M	45	Abdominal collection	Pancreatitis, post-ERCP	*Klebsiella aerogenes*	Blood	4.01	Excluded
27	51	F	60	Pneumonia	Pulmonary embolism	*Klebsiella pneumoniae*	BAL	8.05	
28	91	M	91	Pneumonia	COPD, prostate cancer	ESBL *K. pneumoniae*	BAL	8.97	
29	78	M	70	Subscapular abscess	Hypertension	Gram negative bacilli from stain	Abscess drainage	6.19	Excluded
30	41	F	101	Cellulitis left arm	Obesity, hypertension	*Serratia marcescens*	Tissue sample	12.00	
31	58	F	60	Osteomyelitis left foot	DM, PAD	*Escherichia coli*	Tissue sample	2.94	
32	67	F	59	Cellulitis left breast	Breast cancer, DM	*Klebsiella aerogenes*	Tissue sample	8.22	
Patients with inflammatory and/or oncological disease without infection									
33	64	M	93	Interstitial lung disease	-	-	-	-	
34	60	M	74	Interstitial lung disease	-	-	-	-	
35	59	F	84	Interstitial lung disease	-	-	-	-	
36	54	M	86	Interstitial lung disease	-	-	-	-	
37	64	M	89	Breast cancer	-	-	-	-	

Table notes: Type 2 diabetes mellitus (DM), chronic kidney disease (CKD), acute myeloid leukemia (AML), peripheral artery disease (PAD), endoscopic retrograde cholangiopancreatography (ERCP), bronchoalveolar lavage (BAL), extended-spectrum betalactamase (ESBL), cerebrospinal fluid (CSF), chronic obstructive pulmonary disease (COPD).

Data file S1. Individual subject-level data. (Excel file)

Movie S1. ^{18}F-FDS PET/CT in a SARS-CoV-2-infected hamster. CT (left) and PET/CT overlay (right) of ^{18}F-FDS in a representative animal infected with SARS-CoV-2. ^{18}F-FDS signal is observed in the kidneys, gallbladder and intestine due to normal excretion.

Movie S2. ^{18}F-FDS PET/CT in a SARS-CoV-2 and *K. pneumoniae* coinfected hamster. CT (left) and PET/CT overlay (right) of ^{18}F-FDS in a representative animal infected with SARS-CoV-2 and *K. pneumoniae*.