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Supplemental information

Diverse bacteria elicit distinct neutrophil responses in a physiologically relevant model of infection

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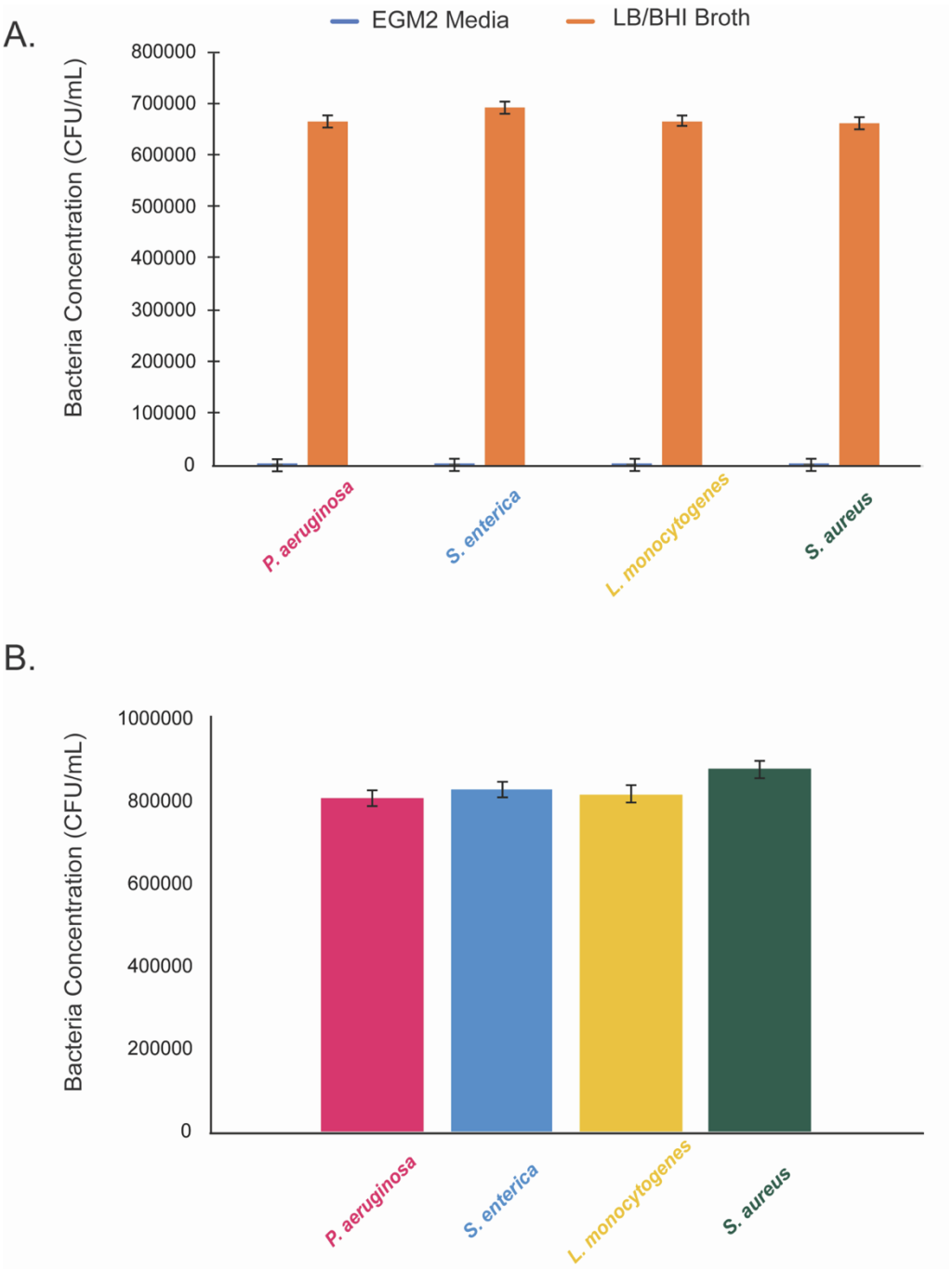

Figure S1: All bacterial species are alive but not dividing over 16 hours, related to Figure 1.

A. Bacterial CFUs when grown for 16 hours in EGM-2 (blue) and either LB broth (for *P. aeruginosa* and *S. enterica*) (orange) or BHI broth (for *L. monocytogenes* and *S. aureus*). Data from 3 independent experiments. Error bars represent the mean plus standard error of the mean (SEM).

B. Bacterial CFUs in the Infection on a Chip device following a 16-hour experiment with neutrophils. Data quantified from 9 lumens across 3 independent experiments. Error bars represent the mean plus SEM.

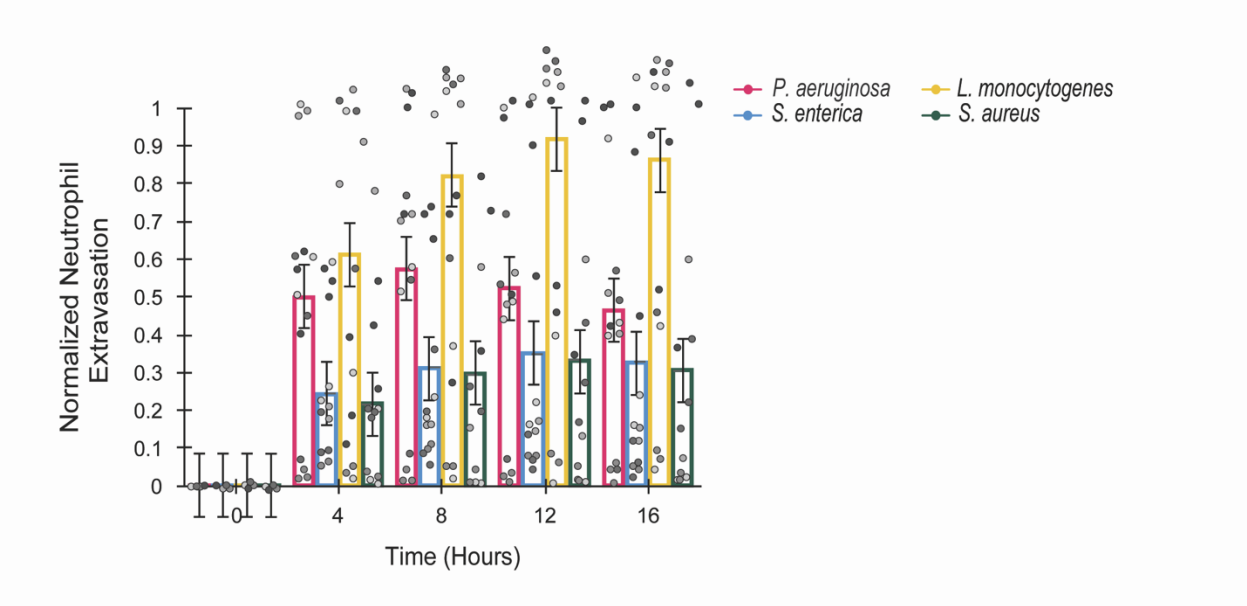

Figure S2: Neutrophils have increased extravasation in response to *L. monocytogenes,* related to Figure 2.

A. The number of neutrophils outside the lumen, normalized to the number of neutrophils initially in the lumen was quantified for *P. aeruginosa, S. enterica, L. monocytogenes,* and *S. aureus* every 4 hours for 16 hours. Data quantified from 14 lumens (*P. aeruginosa*), 13 lumens (*S. enterica*), 14 lumens (*L. monocytogenes*), or 12 lumens (*S. aureus*) across 5 independent experiments. Error bars represent the mean plus SEM. All bacteria were compared to each other at each time point and analyzed with ANOVA. For each condition, emmeans and SEM were calculated and pairwise comparisons were performed with Tukey's adjustment. Asterisks represents significance of neutrophil extravasation for each bacterial species condition compared to *L. monocytogenes* condition. P-values are labeled as *P< .05. This graph displays the same data as Figure 2 but with individual measurements shown. Individual data points are displayed with each gray scale color representing a different replicate.

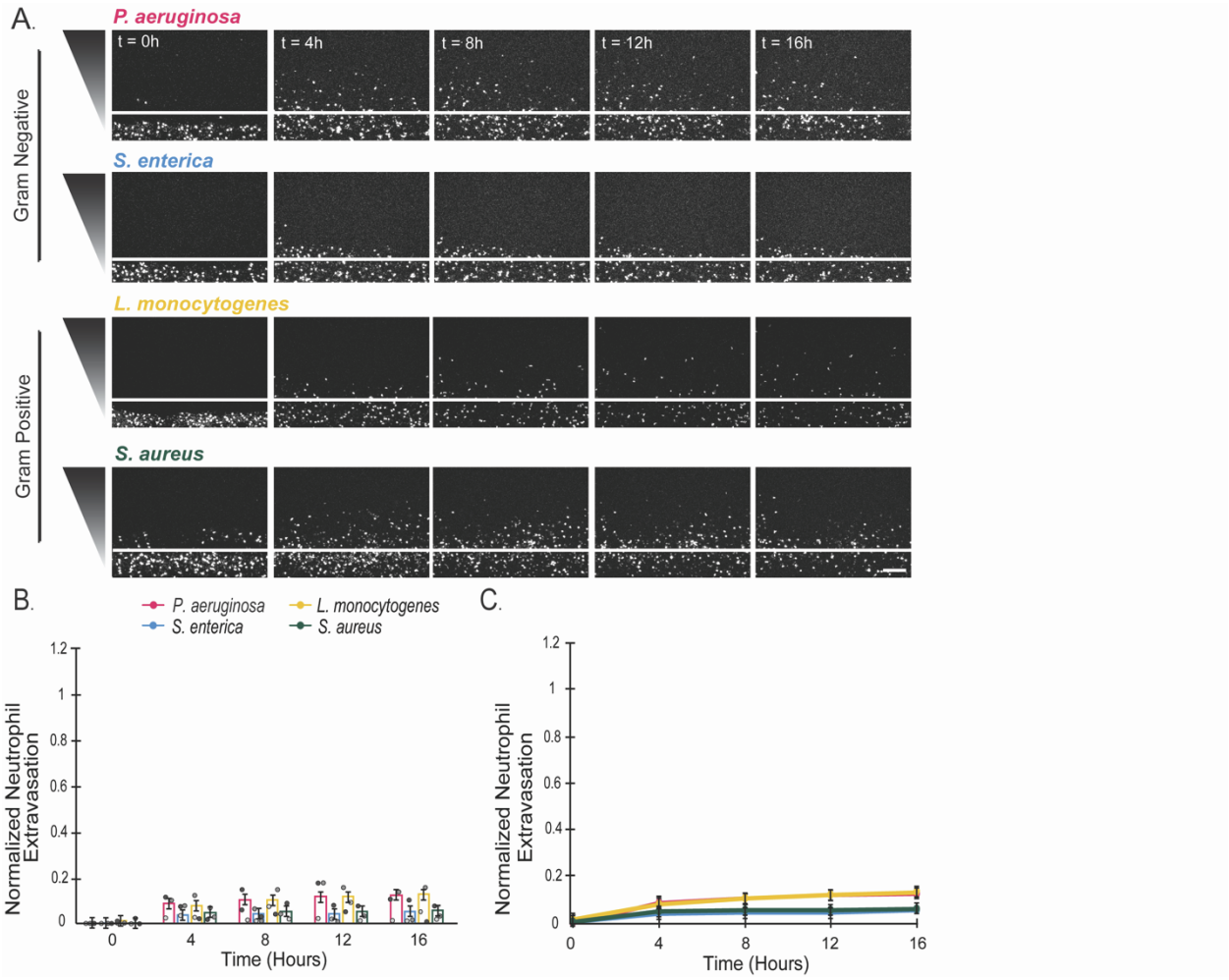

Figure S3: Neutrophils show minimal extravasation in response to all bacterial species without endothelial cells present, related to Figure 2.

A. Representative images of neutrophils extravasating out of lumens, without endothelial cells present, in response to *P. aeruginosa, S. enterica, L. monocytogenes,* or *S. aureus* at 4-hour intervals. Neutrophils stained with Calcein AM (white). White line represents the lumen boundary. Bacterial gradient direction shown on left. Scale bar is 100 µm.

B-C. The number of neutrophils outside the lumen shown in (B)bar graph form and (C)line graph form, normalized to the number of neutrophils initially in the lumen was quantified for *P. aeruginosa, S. enterica, L. monocytogenes,* and *S. aureus* every 4 hours for 16 hours. Data quantified from 9 lumens (*P. aeruginosa*), 9 lumens (*S. enterica*), 9 lumens (*L. monocytogenes*), and 9 lumens (*S. aureus*) across 3 independent experiments. Individual data points are displayed with each

gray scale color representing a different replicate. Error bars represent the mean plus SEM.

	Time (Hours)	Speed	Length	Distance	Straightness
P. aeruginosa	2	0.0035	0.0092	<0.0001	<0.0001
	4	0.0016	0.0028	<0.0001	0.0008
	6	<0.0001	<0.0001	<0.0001	<0.0001
	8	<0.0001	<0.0001	<0.0001	0.0024
S. enterica	2	0.046	0.0195	<0.0001	0.0001
	4	<0.0001	<0.0001	<0.0001	0.0005
	6	0.0607	0.0292	<0.0001	<0.0001
	8	<0.0001	<0.0001	<0.0001	<0.0001
L. monocytogenes	2	0.0025	0.0031	<0.0001	<0.0001
	4	0.015	0.0172	<0.0001	<0.0001
	6	0.0896	0.125	<0.0001	<0.0001
	8	<0.0001	<0.0001	<0.0001	<0.0001
S. aureus	2	0.0193	<0.0001	0.0047	0.9856
	4	0.0007	<0.0001	<0.0001	0.1175
	6	0.0001	<0.0001	<0.0001	0.1186
	8	<0.0001	<0.0001	<0.0001	0.3366

P-Values

>0.05

0.01-0.05

0.001-0.01

0.0001-0.001

<0.0001

Figure S4: P values of interstitial migration characteristics, related to Figure 3. Each interstitial migration characteristic (speed, length, distance, straightness) for each bacterial was compared to the value of the same bacterial species at time zero. Shading indicates level of significance, as labeled in the legend. For each condition pairwise comparisons were performed with Tukey's adjustment.

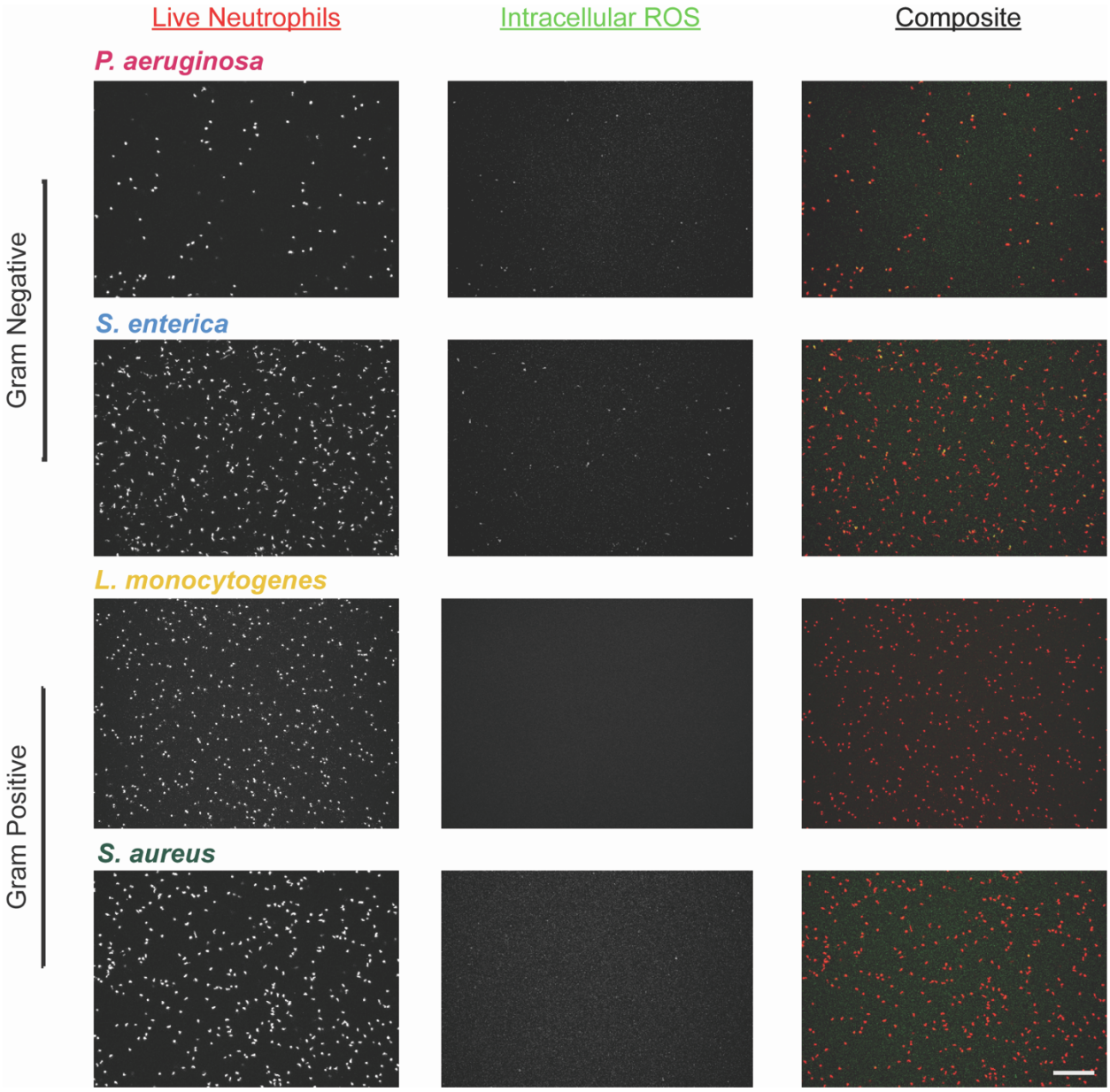

Figure S5: An increased percentage of neutrophils produce ROS in response to Gram-Negative bacteria, *P. aeruginosa* and *S. enterica,* related to Figure 4. Neutrophils were seeded in collagen in well plates and stained with DHR123 in the presence of *P. aeruginosa, S. enterica, L. monocytogenes,* and *S. aureus* to visualize intracellular ROS production. Representative images showing intracellular ROS production (DHR123) and total neutrophils (Calcein AM) in the Infection-on-a-Chip device in the presence of *P. aeruginosa, S. enterica, L. monocytogenes,* and *S. aureus*. Images shown are at 3 hours after introduction of bacteria. The first column shows all neutrophils stained red with Calcein AM, the second column shows DHR123 positive neutrophils, fluorescent ROS producing cells. Scale bar is 100 µm.

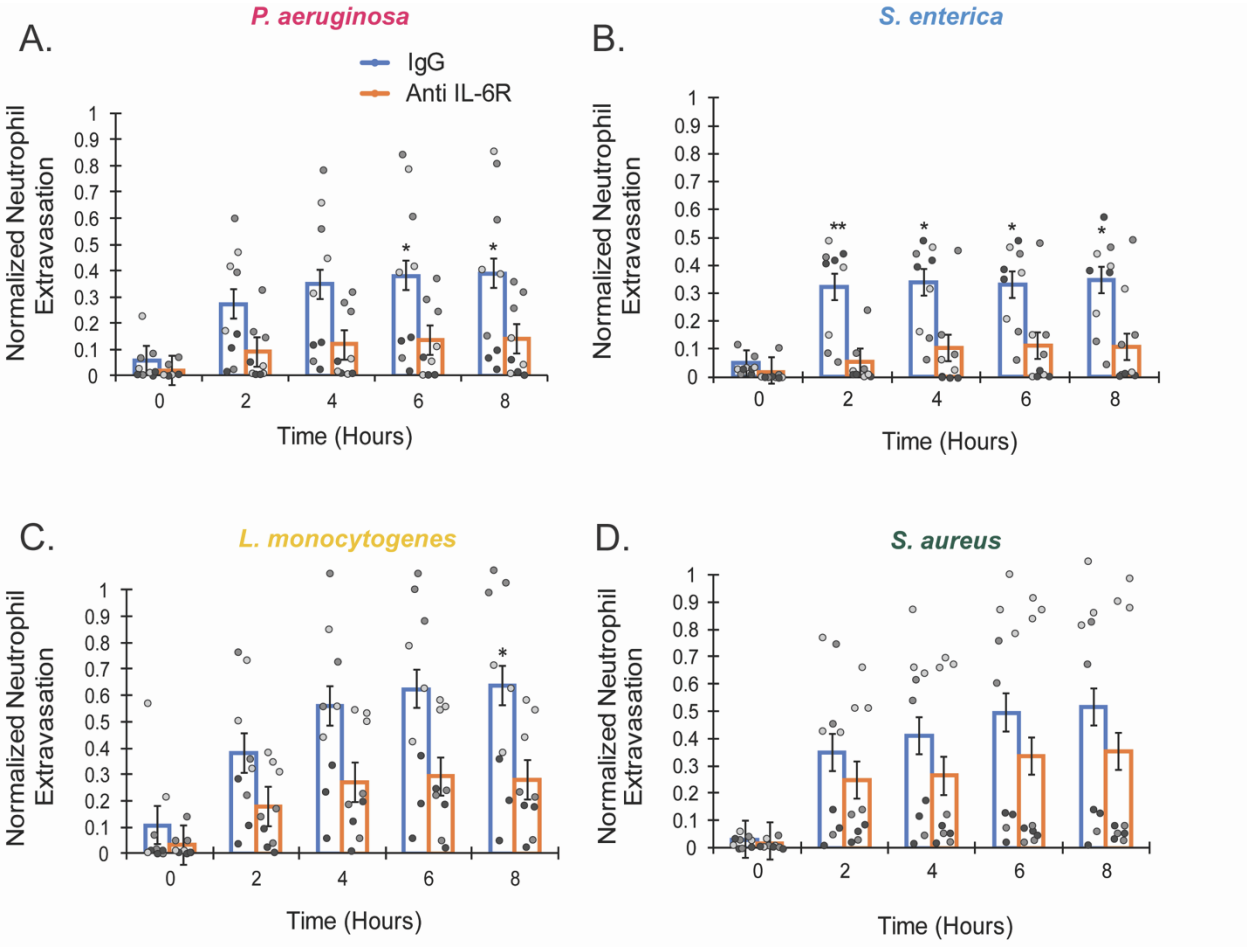

Figure S6: IL-6 is required for neutrophil extravasation in response to diverse bacterial species, related to Figure 6.

A-D. The number of neutrophils outside the lumen, normalized to the number of neutrophils initially in the lumen was quantified for (A) *P. aeruginosa,* (B) *S. enterica,* (C) *L. monocytogenes,* and (D) *S. aureus* every 2 hours for 8 hours in the presence of either an IgG control antibody or IL-6 receptor blocking antibody. Data quantified from 9 lumens for each bacterial species and each antibody condition across 3 independent experiments. Error bars represent the mean plus SEM. All bacteria were compared to each other at each time point and analyzed with ANOVA. For each condition, emmeans and SEM were calculated and pairwise comparisons were performed with Tukey's adjustment. Asterisks represent significance between IL-6 receptor blocking antibody condition and IgG control condition. P-values are labeled as *P< .05;**P<.01. This graph displays the same data as Figure 6 but with individual measurements shown. Individual data points are displayed with each gray scale color representing a different replicate.