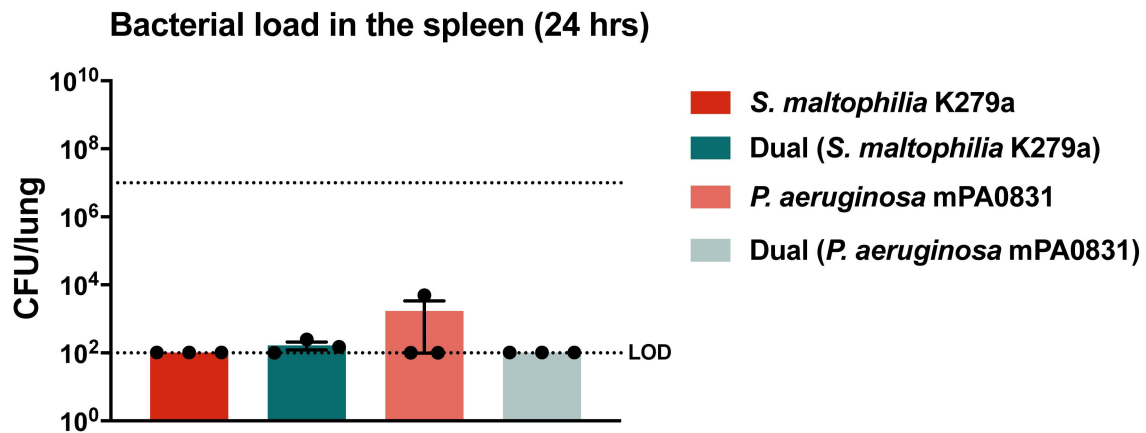


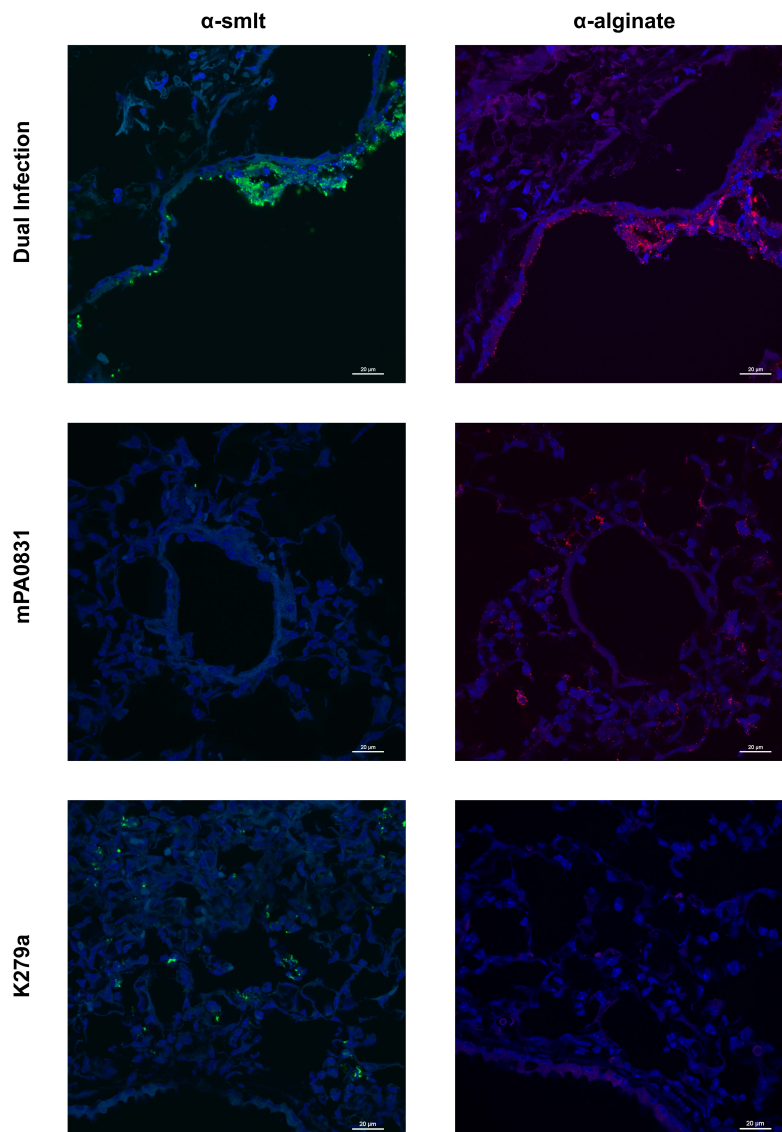
**Fig S1. *S. maltophilia* and *P. aeruginosa* form polymicrobial biofilms *in vitro***

Static single- and dual-species biofilms of *S. maltophilia* K279a and A) *P. aeruginosa* mPA0831 or B) *P. aeruginosa* PAO1, *S. maltophilia* msm2 and C) *P. aeruginosa* mPA0831 or D) *P. aeruginosa* PAO1, and *S. maltophilia* msm4 and E) *P. aeruginosa* mPA0831 or F) *P. aeruginosa* PAO1 were seeded at  $\sim 10^7$  CFU/mL of each organism in LB and grown at 30°C up to 24 hours. Mean  $\pm$  SEM, n = 3. Two-way ANOVA. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001, \*\*\*\* P<0.0001.



**Fig S2. Dual-species infection does not increase bacterial dissemination**

BALB/cJ mice were intratracheally infected with  $\sim 10^7$  CFU of *S. maltophilia* K279a alone, or in the presence of heat-killed *P. aeruginosa* mPA0831 before being euthanized at 24 hours post-infection. A) Bacterial burden in the spleen was enumerated via viable colony counting. Mean  $\pm$  SEM, n = 3. One-Way ANOVA with Tukey's post-hoc comparisons. Groups with undetectable colony counts were represented at the limit of detection (LOD).



**Fig S3. Antibodies used for bacterial staining are efficient and specific**

Representative images of fluorescently stained lung sections were taken via confocal laser scanning microscopy (CLSM). In order to confirm efficacy and specificity of our staining scheme, 10 $\mu$ m serial sections were cut from lungs of mice infected with  $\sim 10^7$  CFU/animal of *S. maltophilia* K279a, *P. aeruginosa* mPA0831, or both in combination 24 hours post-infection. *S. maltophilia* was stained via anti-sera from rabbits immunized with heat-killed *S. maltophilia* (green), and alginate was stained with an anti-alginate polyclonal rabbit antibody (red). Lung structures were visualized with DAPI (blue).

**TABLE S1** Total mortality\* rates of wild-type BALB/cJ mice post-infection

Group	Total (Deaths)	Percent Mortality
Control	29 (0)	0.0
<i>S. maltophilia</i> K279a (~10 <sup>8</sup> )	15 (2)	13.3
<i>S. maltophilia</i> msm4 (~10 <sup>8</sup> )	15 (2)	13.3
<i>S. maltophilia</i> K279a (~10 <sup>7</sup> )	58 (0)	0.0
<i>S. maltophilia</i> msm2	10 (0)	0.0
<i>P. aeruginosa</i> mPA0831	49 (9)	18.4
Heat killed <i>P. aeruginosa</i> mPA0831	12 (0)	0.0
<i>P. aeruginosa</i> FRD1	10 (0)	0.0
<i>P. aeruginosa</i> FRD1mucA+	10 (1)	10.0
<i>S. maltophilia</i> K279a + <i>P. aeruginosa</i> mPA0831	43 (19)	44.1
<i>S. maltophilia</i> K279a + heat killed <i>P. aeruginosa</i> mPA0831	12 (0)	0.0
<i>S. maltophilia</i> K279a + <i>P. aeruginosa</i> FRD1	20 (3)	15.0
<i>S. maltophilia</i> msm2 + <i>P. aeruginosa</i> mPA0831	10 (0)	0.0

\*Mortality is defined as animal death before the conclusion of the study