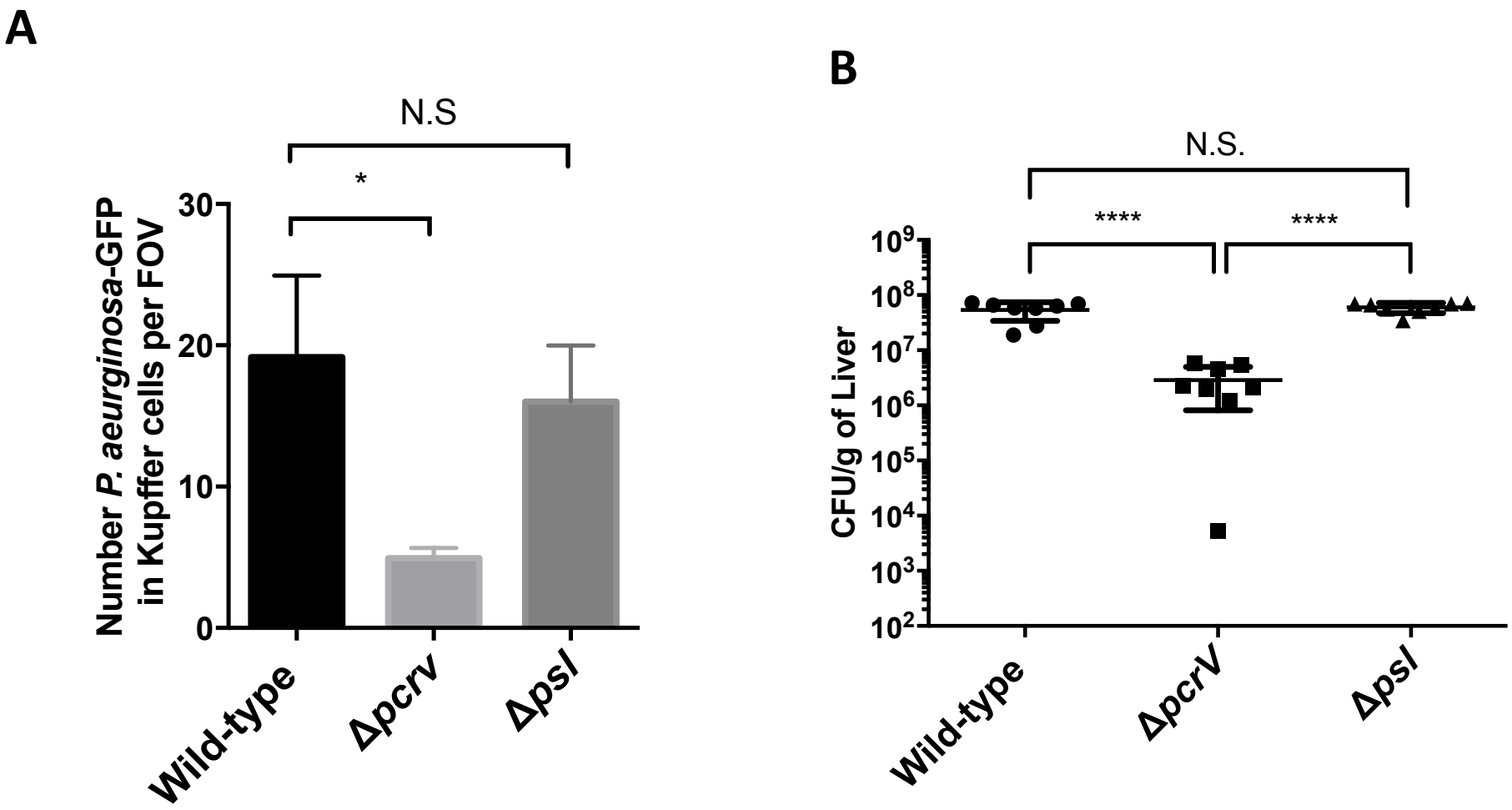
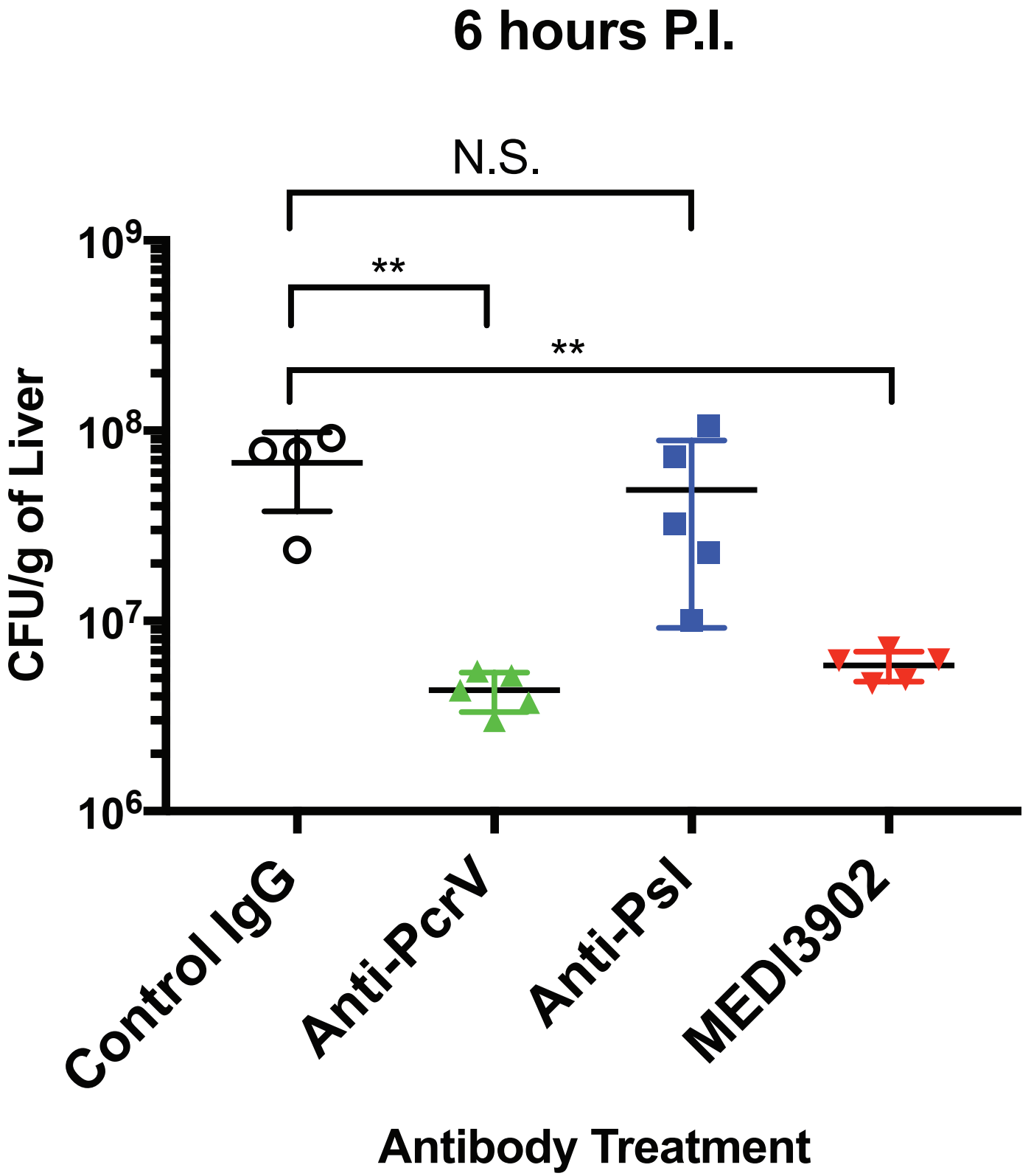
Supplemental Figure 1



Supplemental Figure 1: *P. aeruginosa ΔpcrV* did not persist in the liver as long as wild-type or Δ*pslA* strains. A. Quantification of phagocytosis of multiple *P. aeruginosa* strains by Kupffer cells in the livers of mice at 4 hours post-infection. Intravital images were quantified by determining the number of bacteria arrested on or inside a Kupffer cell per field a view n = 4, 3 FOV per mouse was assessed error bars, SEM, ****p<0.0001. **B.** Bacterial colony forming units (CFUs) were determined at 6 hours post-intravenous infection with *P. aeruginosa*. N=8, error bars represent SEM, *p=0.0158.
One-way ANOVA statistical analysis was performed, multiple comparisons were made for the indicated columns. All experiments were repeated three times unless otherwise indicated.

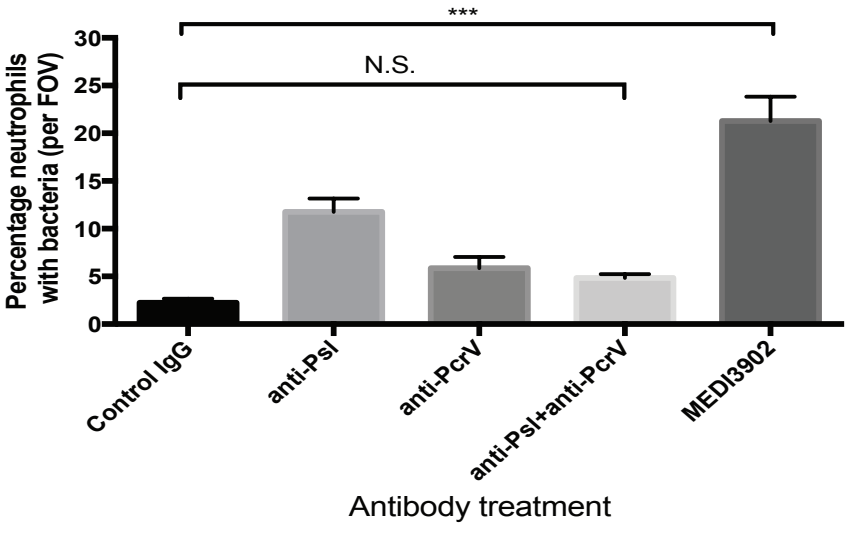
Supplemental Figure 2



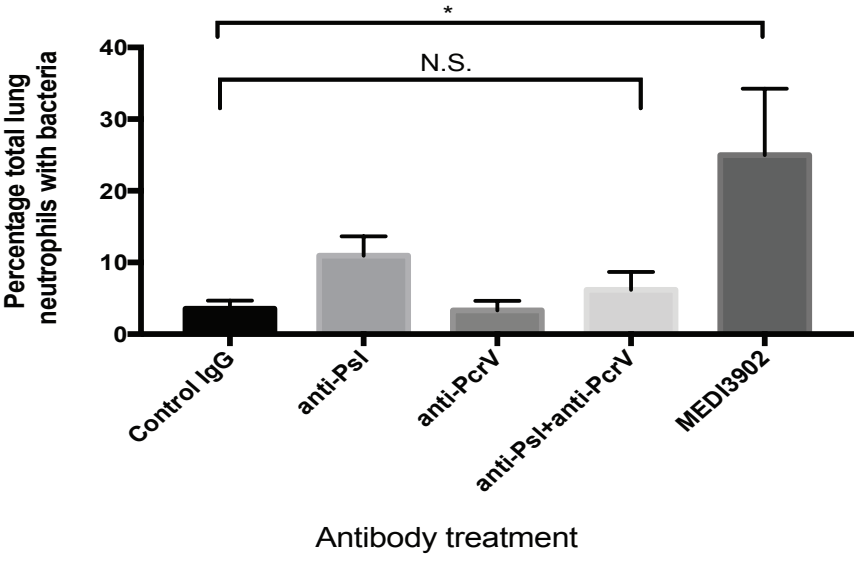
Supplemental Figure 2. Prophylactic treatment of mice with anti-PcrV and MEDI3902 reduced bacteria in the liver at 6 hours post infection. Bacterial colony forming units (CFUs) were determined at 6 hours post-intravenous infection with *P. aeruginosa* wild-type, Δ*pslA* and *ΔpcrV*, N=5, **p=0.0047.
One-way ANOVA statistical analysis was performed, multiple comparisons were made for the indicated columns. All experiments were repeated three times unless otherwise indicated.

Supplemental Figure 3

A

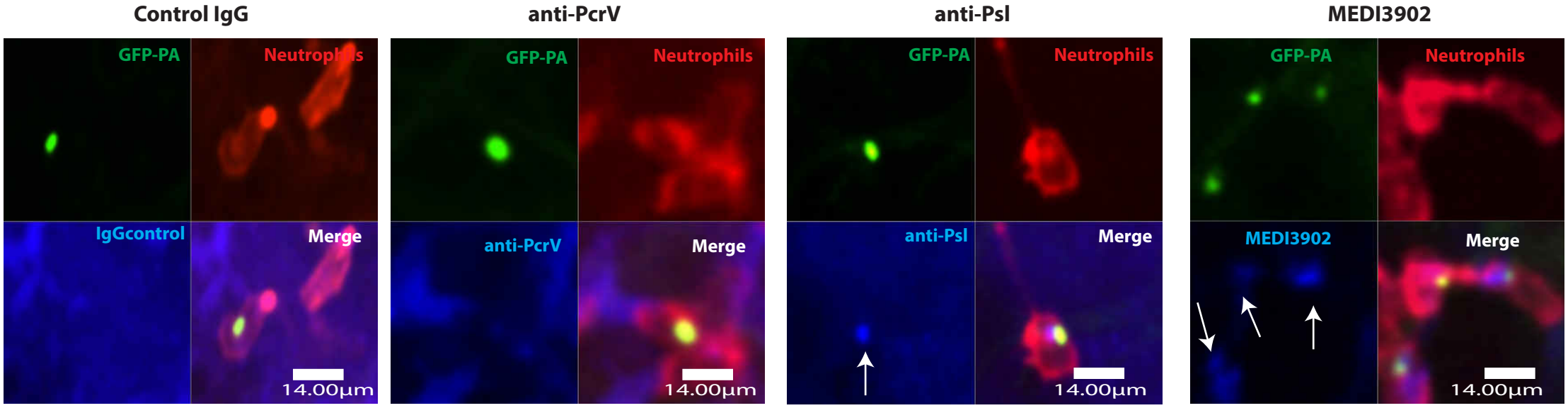


B



Supplemental Figure 3. Mixture of anti-Psl and anti-PcrV do not have the same effect as MEDI3902. Mice were prophylactically treated with 12.5mg/kg anti-Psl and 12.5mg/kg anti-PcrV antibody given in one bolus interperatonially or treated with 15mg/kg of single antibodies. **A.** The number of neutrophils able to phagocytize GFP *P. aeruginosa* in the lung vasculature was assessed using intravital microscopy. Data is represented as the percentage of total number of neutrophils per field of view with bacteria. One-way ANOVA analysis was performed on the indicated columns. Error bars represent standard error of mean. N=3, 3 fields of view (FOV) per mouse was assessed. All experiments were repeated three times unless otherwise indicated. ***$p<0.0002$. **B.** Percentage of neutrophils containing bacteria was assessed using flow cytometry, One-way ANOVA analysis was performed on the indicated columns. Error bars represent standard error of mean. N=3, 3 fields of view (FOV) per mouse was assessed, *$p=0.0220$.

Supplemental Figure 4



Supplemental Figure 4. Anti-Psl and MEDI3902 antibody can readily enter neutrophils. Therapeutic antibodies were labeled with Alexa-647 fluorophores and localized in neutrophils with bacteria. White arrows point to cells containing bacteria that are positive for therapeutic antibody labeling.

Supplemental Video Legend:

Video 1: Rapid uptake of *P. aeruginosa* by intravascular Kupffer cells in the liver. Intravital video of liver vasculature. Time-lapse video of 10 minutes was captured as *P. aeruginosa* (green) was injected into the blood stream of mice in real-time. Kupffer cells are seen in blue and neutrophils are visualized in red using F480 and Ly6G antibodies respectively.

Video 2: Dynamic movement of neutrophils in lung vasculature in untreated mice. Time-lapse video of 10 minutes intravital video of an untreated mouse's pulmonary vasculature. Neutrophils (red) stained with Ly6G clone 1A8, CD31 (blue) to stain endothelial cells. Dark circular spaces are alveolar space.

Video 3: Lung resident neutrophils are unable to recognize blood-borne *P. aeruginosa.* Time-lapse intravital video of 10 minutes of a wild-type C57BL/6 mouse lung vasculature. Neutrophils (red) 30 minutes after IV infection with *P. aeruginosa* 6077 (green), endothelium is visualized in blue. White arrow points to a neutrophil unable to recognize arrested bacteria Video length is 10mins.

Video 4: Lung resident neutrophils are able to recognize blood-borne *Staphylococcus aureus.* Intravital video of a C57BL/6 mouse lung vascular neutrophils (red) 30mins after IV infection with *S. aureus* (green) endothelium is visualized in blue. White arrows distinguish neutrophils phagocytizing *S. aureus*. Video length is 10mins.

Video 5: Lung resident neutrophils are able to recognize a very small number of *P. aeruginosa* when mice are prophylactically treated with anti-PCRV antibody. Intravital video of a mouse lung vascular neutrophils (red) 30 minutes after IV infection with *P. aeruginosa* 6077 (green), endothelium is visualized in blue. Mouse was prophylactically treated with anti-PcrV antibody. White arrow points to a neutrophil recognizing a bacteria but unable to engulf it. Video length is 10mins.

Video 6: Lung resident neutrophils are able to phagocytize *P. aeruginosa* when mice are prophylactically treated with anti-Psl antibody. Intravital video of a mouse lung vascular neutrophils (red) after 30 minutes IV infection with 6077 (green), endothelium is visualized in blue. Mouse was prophylactically treated with anti-Psl antibody. White arrow points to a neutrophil with engulfed bacteria. Video length is 10mins.

Video 7: Lung resident neutrophils are able to phagocytize *P. aeruginosa* when mice are prophylactically treated with bi-specific antibody. Intravital video of a mouse lung vascular neutrophils (red) after 30 minutes IV infection with *P. aeruginosa* 6077 (green), endothelium is visualized in blue. Mouse was prophylactically treated with bi-specific antibody. White arrow points to a neutrophil with engulfed bacteria. Video length is 10mins.

Video 8: Lung resident neutrophils are in fact internalizing bacteria after antibody treatment not just coming in contact with them. 3D reconstruction of intravital video for 10 minutes. Neutrophils are seen in red and *P. aeruginosa* in green. Mouse was prophylactically treated with bi-specific antibody and infected IV with *P. aeruginosa* for 30mins. Video length is 10mins.